FACTORS AFFECTING INCUBATION TIME AND CHICK QUALITY

BY

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With love to

My husband Carlos Miguel Garcia-Bojalil
My daughter Carla Denisse Garcia-Bojalil

My family

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By

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Hatching performance is influenced by a wide spectrum of interrelated factors, that determine its success and length of the incubation period.

Two experiments were conducted to evaluate the effects of strain and age of commercial broiler breeders on the conversion of egg to chick and incubation time. Highly significant differences in egg weight were found among strains in both experiments. After removing the effect of egg weight by covariance analysis, significant main effects and their interactions were found on egg weight at transfer, chick weight at hatch and hatch time in the first experiment, but not in the second. Strain and age effects on egg weight loss were not consistent. Highly significant differences due to strain and age were found for yolk and albumen percentage and yolk:albumen ratio. Shell percentage was significantly affected by strain. Egg characteristics were not related to embryonic mortality.

A series of experiments was conducted to evaluate the effect of cold stress and genotype during incubation on incubation time, embryonic mortality, white blood cell counts of newly hatched chicks, and chick growth up to 2 weeks of age. Eggs were cooled continuously for 12, 24, 36, 48, or 72 hours beginning on day 8, 12, 14, 16 or 18 of incubation. Other eggs were cooled intermittently for 6 hours every 48 hours or 12 hours every 96 hours. A control group was not cold stressed. Incubation time was delayed approximately as long as the time of embryonic cooling. Embryonic mortality was increased under continuous cooling, but not under the intermittent cooling for 6 hours. A significant genotype interaction was found in respect to response of embryos of various strains to cold stress. Continuous cold exposure for 36 hours or longer had detrimental effects, both during incubation and after hatch. Total white blood cell counts of chicks exposed to cold for 48 hours were decreased indicating possible immune system compromise.

These studies indicated differences in incubation time due to genotype and breeder age. Strains also differed in their response to embryonic cold stress.

CHAPTER 1

INTRODUCTION

The success of any hatchery depends on the number of high quality chicks obtained from the total eggs set for incubation. To obtain the largest number of chicks with the best quality, adjustments in incubation procedures are required, given that modern birds have changed and improved over the years as a result of a better understanding about genetics, physiology, nutrition, and management.

The development of the embryo after oviposition and from the beginning through all the incubation process until the chick emerges from the shell, is the result of many physiological processes. Their successful occurrence, such as weight loss from the egg, oxygen and carbon dioxide exchange through the shell, and metabolic processes, determine the viability of the embryo, length of incubation, chick weight at hatch, and its posthatch performance. The occurrence of these physiological processes may be altered by environmental conditions in the incubator, egg characteristics, age and genotype of the parents, or by the embryo itself.

The embryo is very sensitive to environmental conditions of temperature during incubation because it is not able to regulate its body temperature to ensure the physiological processes required for its successful development.

Thus, extremes high or low temperatures, or prolonged exposure to these temperatures during embryonic development, may lead to deleterious effects on embryo development, which may in turn affect its posthatch performance. Similarly, length of the incubation period is strongly influenced by temperature. Slightly high temperatures decrease incubation time, while slightly low temperatures increase incubation time. These effects are the result of interactions of many factors, including egg weight, which interacts with shell characteristics and genetic factors to determine chick weight at hatch.

One of the most important advances due to artificial selection is the rapid growth of commercial strains of broiler. As a correlated response to selection for body size, egg size has been increased. This might result in changes of physical qualities of the shell, amount of water loss by diffusion across it, relative proportions of egg components, as well as embryo physiology and duration of the incubation period that may be different among strains.

Recently, continuous or intermittent cold stress during embryonic development has been suggested as a tool to improve hatchability and growth characteristics of the chick. However, few studies indicate the effects on weight loss, chick performance after hatch, delay of the incubation process, or disturbances on the immunological system which seems to be affected by low temperature.

Considering the common practice in commercial hatcheries of holding the chicks in the hatcher up to 21.5 days of incubation to ensure maximum hatch of fertile eggs, it is very important to determine if there are variations in hatch time due to genotype and environmental factors, and how new procedures of incubation may help to approach optimum hatch and posthatch performance.

With this in mind, the current study was designed to determine if commercial strains of broilers breeders at various ages differed in egg and chick characteristics during incubation, and how these differences, if any, might affect the length of the incubation period. Furthermore, the effects of short term cold stress during incubation on embryonic viability, chick weight, posthatch white blood cell profile, and growth performance of chicks cooled during incubation were tested.

CHAPTER 2
LITERATURE REVIEW

The success or failure of a fertile egg to hatch is influenced by two complex factors. The first is related to genetics, nutrition and health of the breeder, oviductal time, management of the egg prior to incubation, and environmental condition in the incubator. The second one is determined by the physiology of the embryo, which enables the embryo to take the nutrients required for its growth from the yolk, albumen and shell and gas exchange. The combination of these two factors determines the number of chicks successfully hatched, the length of the incubation period and the mortality due to malpositions, malformations or other causes.

Several factors involved in embryo development during incubation have been identified. This review will focus on factors affecting chick weight, weight loss from the egg, length of incubation, egg components, and the effect of low temperature during incubation on embryonic development, immune system and growth of the posthatch chick.

Chick Weight

Chick weight is determined by egg weight at set (Wilson, 1991a), weight loss from the egg during the incubation period, and weight of the shell and membranes (Tullet and Burton, 1982). Also, age and genotype of the breeders

(Bray and Iton, 1962; Sherman and Shults, 1989) and incubator conditions (Peebles *et al.*, 1987) play important roles.

Wilson (1991a), in an extensive review about the interrelationships of egg size, chick size and hatchability, indicated a strong positive correlation between egg weight and the weight of the chick hatched from it. Shanawany (1987), in agreement with others researchers (Whiting and Pesti, 1983; Reinhart and Hurnik, 1984; Shanawany, 1984; Yannakopoulos and Tserveni-Gousi, 1987), established that chick weight at hatching is a linear function of egg weight at setting, with a highly significant and positive correlation. Although this correlation does not exist during the first half of the incubation period, it is high at the time of hatching (Bray and Iton, 1962). It is possible that larger eggs produce heavier chicks at hatch, either by having a large carcass and smaller yolk sac, or by having a less developed carcass and larger yolk sac (Wilson, 1991a). Skewes *et al.* (1988) indicated an increase of both carcass weight and residual yolk sac weight in bobwhite quail from large eggs, with significant differences in chick weight, but these differences were not significant when chick weight and yolk sac weight were expressed as percentages of egg weight and chick weight.

Genetic effects on chick weight as percentages of egg weight are not clear. Jull and Heywang (1930) indicated individual effects of the hen on egg weight-chick weight ratio. Whiting and Pesti (1983) found differences in chick weight between dwarf broiler breeders and a standard strain when chick weight was expressed as percentage of egg weight. The age of the breeder seems to be an important factor determining the weight of the embryo at hatch. This effect is greater for young breeders (Pone *et al.*, 1985; Wilson, 1991b) and could be due to a direct relationship between hen age, hen body weight and egg size. However, Shanawany (1984) indicated that as parental age increases, the embryo weight increases, regardless of egg weight. According to this researcher, most of the increase of embryo weight occurs between 28 and 36 weeks of age of the breeder with no significant effect thereafter. Yannakopoulos and Tserveni-Gousi (1987) found a linear increase of egg weight and chick weight until 52 weeks of age. However, when chick weight was expressed as percentage of egg weight this response was curvilinear. Wilson and Harms (1988) found that chick weight was related to egg weight and age of the parents. Results obtained by Reinhart and Hurnik (1984) indicated highly significant effects of age of the breeder on chick weight.

Chick weight at hatch is mainly determined by egg weight, which may be affected by age and genotype of the parents, and weight loss during incubation.

Weight Loss

Weight loss from the egg is due to loss of water. Loss of water is partially determined by the porosity of the shell and seems to be affected by genotype of the animals (Landauer, 1967). Buss (1982) indicated differences between families, strains and breeds of chickens in shell formation. He concluded that quality of the shell can be altered toward better or poorer quality by selection. Anthony *et al.* (1989a, 1989b) reported significant differences in shell weight, specific gravity and shell thickness between lines selected for high and low body weight. Similar results, regardless of body weight, had been indicated by Arafa *et al.* (1982) in five different strains of commercial laying hens. Lewis and Perry (1987) reported significant genotype effects on both shell weight and shell density.

Rate of loss from the egg during incubation have been directly related to rate of embryonic development (Rahn and Ar, 1980), prepipping O_2 consumption rate (Ar and Rahn, 1980), metabolic rate (Burton and Tullet, 1983) and shell conductance (Rahn and Ar, 1974), but inversely related to incubation time (Rahn and Ar, 1974; Hoyt, 1980).

Some reports emphasize the importance of regulation of water loss from the egg. Sufficient water loss is a requirement during incubation to maintain the water content essentially the same as at the beginning (Ar and Rahn, 1980), to ensure air cell formation for the support of pulmonary respiration (Rahn and Ar, 1974). Nevertheless results of studies on weight loss and their effects on hatchability have been conflicting. McDaniel *et al.* (1979) found that eggs with the greatest weight loss had the lowest hatchability. Mier *et al.* (1984) established that turkey eggs with weight losses below 10% and above 15% had significantly lower hatchability than those at 12%, which had the highest hatchability.

Incubation Time

The factors affecting incubation time have been examined in great detail by Wilson (1991a). Hatch time is correlated positively with egg weight (Burton and Tullet, 1985). Rahn and Ar (1974) indicated that incubation time is proportional to egg weight raised to the power of .22. Hassan and Nordskog (1971) indicated correlation from -.17 at 8 days to .90 at hatch between embryo and egg weight, with a significant effect due to strain. Variations in hatch time due to age of the female (Crittenden and Bohren, 1962; Smith and Bohren, 1975), breed and line within breed (Smith and Bohren, 1975) and pre-incubation conditions may occur. Pre-incubation storage time and conditions affect much more the length of incubation than egg size (MacLaury and Insko, 1968; Bohren, 1978; Wilson, 1991a).

Results of genetic studies on the relationship of body weight and egg weight with hatch time are inconsistent. Crittenden and Bohren (1961) indicated a heritability of .49 for hatch time in agreement with Siegel *et al.* (1968), who reported heritability of .50. However, conflicting results are found in the literature relative to correlated response on hatch time. A genetic correlation of .20 between hatch time and egg weight was reported by Crittenden and Bohren (1961), while Siegel *et al.* (1968) indicated a genetic correlation of .11 and a negative phenotypic correlation of -.32 between body weight and hatch time. On the contrary, Smith and Bohren (1974) indicated no evidence of genetic correlation between hatch time and egg weight.

Incubation temperature affects the normal development of the embryo and consequently the duration of the incubation period. This effect is dependent on the temperature itself (slightly high temperature decreases incubation time, while slightly low temperature increases incubation time), age of the embryo and duration of exposure. As a general rule, it has been established that hatch time is delayed approximately the same as the hours of cooling used. Taylor *et al.* (1933) indicated that chilling eggs by interruption of the incubator power supply for 12 hours resulted in a hatch period postponed 18 to 20 hours behind the control group. However, this was not true for eggs chilled on day 21 of incubation. Similar results related to delayed hatch time due to lower temperature than the temperature established for normal incubation were reported by Booth and Rahn (1991), who indicated that cooling eggs for a total of 126 hours (6 hours every day) at 24 C, delayed hatch time 170.4 hours. Decuypere and Michels (1992) reported a delay of only 24 hours in hatch time for eggs cooled for 54 hours (6 hour every day from day 11 to 20 of incubation).

Many factors are involved or interact to determine the duration of incubation. Among these factors, egg weight, age and genotype of the breeders, and temperature seem to play important roles.

Egg Composition

The egg constitutes the bridge between succeeding generations of chickens. The fertilized egg not only carries the hereditary units that determine the make-up of the chick to be hatched, but also the nutrients that will support the chick during its embryonic development. Differences in egg weight among various lines of chickens have been attributed to differences in yolk, albumen and shell weight (Marion *et al.*, 1964; Cherry *et al.*, 1978; Benoff and Renden, 1983).

Causal factors that influence egg weight are yolk, albumen and shell weight (Cherry *et al.*, 1978). A high genetic correlation exists between yolk and albumen weight and egg weight (Rodda *et al.*, 1977). Most of the variation in egg weight may be accounted for by variation in albumen weight (Cherry *et al.*,1978; Strong and Nestor, 1980; Benoff and Renden, 1983). The larger eggs generally contain greater absolute amount of all components and relatively less yolk and more albumen than the smaller eggs (Hafez *et al.*, 1955; Marion *et al.*, 1964) but the relative amount of shell is the same in eggs of all sizes (Hafez *et al.*, 1955). This may be due to an increase in the weight of albumen with no change in yolk weight resulting in a decreased percentage yolk (Strong and Nestor, 1980; Nestor *et al.*, 1982). Cherry *et al.* (1978) found no significant differences in percentage of yolk and albumen between dwarf or normal weight and low weight lines. In another study with the same populations, the percentage yolk of large eggs from the normal weight line was greater than those from the low weight line. Strain differences in yolk percentage within young flocks of laying hens were reported by Carey (1988). These effects were quite different within the older flock. A significantly low percentage for yolk weight in a strain of Leghorn was reported by Washburn and Marks (1985).

The weight of the egg and its components is influenced by genetic and nongenetic factors. These factors may have a direct effect on egg weight or may affect it indirectly by affecting body weight. Kinney (1969) indicated a high phenotypic and genetic correlation between body weight and egg weight. Significant strain differences in egg components and yolk percentage have been found (Arafa *et al.*, 1982; Carey, 1988). Rodda *et al.* (1977) reported significant differences between strains selected for high egg production and unselected controls in albumen percent solids and in percent albumen of the whole egg, but no strain effects on the yolk percent solids. Similar results were found by Akbar *et al.* (1983) working with White Leghorn hens selected for high egg production and their reciprocal crosses. The genetic effect was significant for yolk and albumen weight expressed on wet and dry bases. The same significant effect was observed for shell weight. Further, these workers found a significant effect of age on protein levels of the yolk, but this effect was not significant for shell weight.

Significant variations in yolk to egg weight ratio and yolk to albumen ratio have been reported (Erasmus, 1954; Rodda *et al.*, 1977). Recently, Hussein *et al.* (1993) indicated differences in yolk to albumen ratio between commercial layer and broiler breeder eggs. Hagger and Marguerat (1985) suggested that better efficiency in layer hens may be obtained by selection for a lower yolk:albumen ratio but the improvement would be very small. Akbar *et al.* (1983) observed significant effects of strain in dry yolk:albumen ratio and percent of solids of the albumen. Reciprocal effects for percentage of dry albumen and albumen:yolk ratio were observed by Anthony *et al.* (1989b). They indicated that a greater yolk dry matter content was observed in a line selected for high body weight versus a line selected for low body weight.

Egg components such as yolk and albumen weight and yolk:albumen ratio are influenced by egg weight, which may be related to body size of the hen, although genetic effects are involved, independent of egg weight. These effects seem to be minimized in shell weight.

Factors Affecting Embryo Development

The development of the embryo at oviposition is variable (Sturkie and Williams, 1945; Romanoff and Romanoff, 1972). This may be due to genetic effects (Siegel, 1962) as well as parental age (Decuypere and Michels, 1992). Some genetic lines lay eggs at an earlier stage of blastoderm development which could be correlated to differences in body temperature or oviductal transit time, or it may be due to an inherent difference in developmental impetus (Decuypere and Michels, 1992). These may affect the development of the embryo during incubation.

The most important factors affecting embryonic development during incubation are temperature, humidity, gaseous environment, egg turning and egg orientation. Except for gaseous environment, which is mainly determined by the porosity of the shell, these factors can be relatively controlled by man (Wilson, 1991b).

Incubation temperatures affect the normal development of the embryo and its posthatching performance. These effects are dependent on the temperature itself, above or below the normal incubation temperatures (Wilson, 1991b).

The hypothermia effect on developing embryos is described in terms of physiological zero temperature, which according to Webb (1987) is the developmental physiological temperature at which all physiological processes do not come to a halt. When the environmental temperature is on the lower side of the normal incubation temperature down to physiological zero (approximately from 27 to 35 C), a disproportionate development usually occurs. It appears that tissues may have different physiological zeros, thus resulting in a synchronous growth (Wilson, 1991b). This suggestion is supported by calculations of Decuypere and Michels (1992) of physiological zeros for eggs exposed to different cooling stresses at various times during the incubation period. Although the estimated values for physiological zeros were in the range of those reported in the literature, they were dependent on the total average of incubation time and differential of temperatures. Lundy (1969) suggested that cooling below physiological zero is less deleterious to the embryo than cooling to a higher temperature at which development may proceed aberrantly. Hypothermia seems to result in a wide variety of developmental abnormalities, but these generally occur after prolonged exposure. It was reported that cerebral damage and somite fusion were produced in embryos of chickens after exposure to temperature of 25-27 C for 48 hours at day 2 of incubation (Azoubel, 1974), but no deleterious effects were detected by Tazawa (1973) in embryos exposed to 30 C for 2 hours daily from day 10 to 18 of incubation. The sensitivity of the embryo to cold stress varies with age and temperature regimen. As embryonic age increases, the sensitivity of the embryo to hypothermia increases (Wilson, 1991b). However, Webb (1987) indicated that high mortality occurs at much younger ages in embryos exposed for 40 hours to 5 or 10 C.

Incubation at 32.5 C has been shown to cause enlarged heart, probably due to a hemodynamic response to circulatory problems (Wilson, 1991b). This effect may depend on the age of the embryo. Tazawa and Nakagawa (1985) indicated that heart rate decreased 30% in 12 - 16 day old embryos, and 25% in older (17 - 18 day old) embryos after 10 minutes of abrupt exposure to 26 - 28 C environment. In the younger embryos, both the systolic and diastolic pressure begin to decrease immediately after exposure to low temperature. As embryos developed, the systolic pressure remains unchanged during at least 1 hour of exposure and afterwards decreased slightly, but the 18 day old embryos retained the systolic pressure unaltered even after low temperature exposure. Tazawa and Rahn (1986) reported that heart rate of 10 day old embryos exposed to 28, 18, and 8 C changed little from 10 to 18 days in all groups, but the time for onset of arrhythmias and cardiac arrest was shortest for embryos exposed to 8 C. However, when the embryos were rewarmed, most of the embryos exposed to 28 and 18 C did not recover their heart beat, but all the embryos exposed to 8 C recovered it, although their tolerance time after cardiac arrest was variable. They concluded that the younger the embryo, the longer its tolerance to cardiac arrest. Embryos cooled to 28 C or near physiological zero temperature, will go into a condition similar to torpor from which they can fully recover depending on the length of torpor, age and temperature (Tazawa and Rahn, 1987).

Beneficial effects on hatchability by cooling broiler breeder eggs during incubation have been reported by Sarpong and Reinhart (1985). They indicated an increase in hatchability due to cooling stress of 22 C for 24 hours on day 16 of incubation suggesting that cooling stress reduces the metabolic rate of the embryos and relieves the stress caused by excessive production of metabolic heat. The contrary was established by Tazawa *et al.* (1988) who indicate that the rate of heat loss during cooling stress exceeds the embryos maximum rate of heat production by at least 6-fold. Geers *et al.* (1983) reported that embryos incubated at 35.8 C from days 1 to 10 and 37.8 from day 11 to hatching, showed compensatory growth and higher heat production than embryos incubated at 37.8 C during all the incubation period. These conditions persisted posthatch up to sexual maturity. Lancaster and Jones (1988) found no beneficial effect on hatchability when cooling 16 day old embryos for 48 hours at 22 C, although chick quality declined with cooling periods longer than 30 hours. Temperatures below 21 C were detrimental to hatchability but cooling embryos to 26.7 C for 24 hours on day 16 of incubation did not have an effect on hatchability.

The effects of low temperature on hatchability may be mediated by genotype of the animals under consideration. Decuypere and Michels (1992) found that embryos from broiler strains are more sensitive to temperature fluctuations, both at the upper and lower limits of incubation temperature, than embryos from layer strains. These differences are also within strains and are dependent on the selection objectives, which may affect tolerance limits for temperature or other factors during incubation through a genetic covariance between traits (Decuypere and Michels, 1992).

Effects of cold stress to the embryos during incubation appear to be linked to posthatching performance of the chickens. A beneficial effect on growth has been indicated, possibly because of the elimination of the weaker chicks (Buckland, 1969), or because of a compensatory growth effect, which is characterized by a higher rate of cell hyperplasia and may persist up to the reproduction period (Geers *et al.*, 1983). However, Kuhn *et al.* (1982) observed a greater posthatch weight loss from cold stressed chickens and Moreng and Bryant (1954) indicated slower growth and high mortality the first 6 weeks posthatching for chicks cold stressed during incubation. Sarpong and Reinhart (1985) found no effect on subsequent chick growth.

Embryo development during incubation is influenced by its development at oviposition and temperature during incubation. The latter is dependent on the temperature per se, age of the embryo and duration of exposure. Exposure to low temperature may affect growth of the embryo, weight loss, metabolic rate and circulatory system, as well as subsequent growth of the hatched chick. These effects are modified by genotype of the animals.

Effect of Low Temperature During Incubation on the Immune System

Thermal stress during the incubation period affects the normal development of the avian embryo altering its metabolism (Tazawa *et al.*, 1988), heart rate (Oppenheim and Levin, 1975; Tazawa and Nakagawa, 1985; Tazawa and Rahn, 1986), hormone production (Decuypere, 1979; Decuypere *et al.*, 1981, 1983; Kuhn *et al.*, 1982) and immune system including the leukocytes profile (Bednarczyk and Coudert, 1984). Allsep *et al.* (1992) indicated that white blood cells were decreased in turkey embryos cooled for 1 hour at 18 C from day 5 to 21 of incubation, while Branislav *et al.* (1978) suggested that a precocious onset of peripheral white blood cells (WBC) may occur due to cold stress during incubation. In the avian embryo, lymphoid organs and differentiation and proliferation of certain leukocytes may be influenced by neuroendocrine functions and environmental modifications during its embryonic development (Branislav *et al.*, 1978; Glick, 1984).

In adult chickens, the effect of thermal stress on the immunological system is well documented. High temperatures have suppressive influence on antibody production (Thaxton *et al.*, 1968; Subba Rao and Glick, 1977; McFarlane *et al.*, 1989) while low temperatures enhance the humoral immune response, possibly due to cold induced changes in the metabolic activity of antigen reactive cells and antibody producing cells (Subba Rao and Glick, 1977). This response may be affected by genotype of the animals (Jeffery *et al.*, 1980). The thymus and bursa of Fabricius functions are interrelated with the neuroendocrine system (Glick, 1984). Thyroid hormones regulate and amplify lymphocyte proliferation (Keast and Ayre, 1980). Adrenocorticotropin (ACTH) has an immunosuppressive effect (Thaxton *et al.*, 1968; Thaxton and Siegel, 1973), producing a relative reduction of lymphocytes and eosinophils and a relative and absolute rise of heterophils (Glick, 1958; Hublé, 1955). In recent research by Allsep *et al.* (1992), turkey embryos cooled for 1 hour at 18 C at different ages showed a significant decrease of circulating heterophils, lymphocytes and basophils, which was reflected in a significant reduction of total WBC.

Conflictive results have been reported for the effects of cold stress during avian embryonic development on later life. A decreased antibody response of animals incubated at low temperature has been indicated (Preda *et al.*, 1972), with important structural and morphological modifications of the lymphoid organs, mainly the bursa of Fabricius and thymus, due to modification of their enzymatic activities (Preda *et al.*, 1971). They indicated a lower antibody response of 15 weeks old chicks incubated at 37 C, compared with chicks of the same age incubated at 38.5 C. However, chronic cold exposure to 7.2 and 24 C of chickens from the day of hatch to 4 weeks of age increased the antibody response in a study reported by Subba Rao and Glick (1977).

Modulation of the immune response during extreme changes in temperature may be a function of hormonal mechanisms. The avian immune system is very sensitive to glucocorticoids (Glick, 1984). In adult chickens, the stress by exposure to extreme low temperature causes a decrease of adrenal cholesterol (Perek and Bedrak, 1962) and adrenal corticosterone (Nir *et al.*, 1975). Adrenal cholesterol has been considered a precursor of adrenal corticosteroids (Sayers, 1950), which are known to be released in the presence of ACTH (Resko *et al.*, 1964) and to produce lymphopenia and heterophilia in the chicken (Glick, 1958). Corticosterone, the principal corticosteroid in birds (Holmes and Phillips, 1976), is synthesized from cholesterol via an ascorbic acid sensitive pathway (Harvey *et al.*, 1984). Exogenous ACTH increases the incorporation of endogenous corticosterone into bursal lymphoid cells and causes lymphopenia (Gould and Siegel, 1981). In the avian embryo, lymphoid organs and differentiation and proliferation of certain leukocytes can be influenced by neuroendocrine functions and environmental modification during embryonic development (Branislav *et al.*, 1978; Glick, 1984). The thymus and bursa of Fabricius (primary lymphoid organs in the young chick) reach their maximum sizes after hatch (Glick, 1977; Kendell, 1980); therefore there is possibly an increased susceptibility of these organs to environmental modifications.

Low temperature may influence the immunological response of the embryo and posthatch chicks. This influence is mediated by neuroendocrine functions of the embryo possibly involving glucocorticoids.

CHAPTER 3
STRAIN AND AGE OF THE FEMALE EFFECTS ON CHARACTERISTICS OF BROILER BREEDER EGGS DURING INCUBATION

Introduction

The weight of the chick at hatch is affected by several factors including species, egg nutrient levels, egg environment, egg size (Wilson, 1991a), weight loss during the incubation period, weight of the shell and other residues at hatch (Tullet and Burton, 1982), as well as shell quality and incubator conditions (Peebles *et al.*, 1987). At the time the chick is removed from the incubator, its weight is determined by the weight at hatch and the time the chick is held in the incubator (Wyatt *et al.*, 1985).

Shanawany (1987), in agreement with other researchers (Whiting and Pesti, 1983; Reinhart and Hurnik, 1984; Shanawany, 1984; Yannakopoulos and Tserveni-Gousi, 1987), indicated that chick weight at hatch depends upon a linear function of egg weight at setting with a highly significant and positive correlation between both variables. This researcher suggested that hatch weight can be predicted using egg weight to the .90 power as a component of the prediction equation. However, it may be affected by variation in water loss during incubation (Tullet and Burton, 1982).

Wilson (1991b), in an extensive review about the relationship of egg size and chick size, indicated a strong positive correlation between egg weight and the weight of the chick hatched from it. The correlation between egg weight and embryo weight is near zero at the beginning of the incubation (Hassan and Nordskog, 1971; All-Murrani, 1978), but as embryo development proceeds this correlation increases to a maximum at hatch (Bray and Iton, 1962), possibly because of a higher assimilation rate of the yolk by the older embryo (Jull and Heywang, 1930).

Factors such as seasonal effects (because of changes in maternal metabolism), genotype, incubation period (Wilson, 1991b), body weight and age of the hen (Benoff and Renden, 1983; Tserveni-Gousi, 1987), as well as correlated responses due to genetic selection (Rodda *et al.*, 1977; Akbar *et al.*, 1983; Fletcher *et al.*, 1983) may alter this egg weight-chick weight relationship.

When considering eggs of the same size, the age of the parents has a curvilinear effect on the weight of the embryo, but this effect does not exist on chick weight at hatch (Shanawany, 1984; Tufft and Jensen, 1991).

In commercial conditions of incubation, the chicks are removed from the hatcher approximately at 21.5 days of incubation to ensure maximum hatch of the fertile eggs. This practice results in a longer holding period in the hatcher that may negatively affect the growth performance of the early hatching chicks (Fanguy *et al.*, 1980; Hager and Beane, 1983; Wyatt *et al.,* 1985).

Hatch time is influenced by many factors. It is correlated positively with egg weight (Burton and Tullet, 1985), with some variation due to age of the hen (Crittenden and Bohren, 1962; Smith and Bohren, 1975), breed, line within breed (Smith and Bohren, 1975; Wilson, 1991b), and pre-incubation storage time (MacLaury and Insko, 1968; Bohren, 1978). This characteristic is heritable

(Crittenden and Bohren, 1961; Siegel *et al.*, 1968) and suitable to be changed by artificial selection (Smith and Bohren, 1974). Siegel *et al.* (1968) indicated that selection for increased body weight at 8 weeks of age resulted in a positively correlated genetic response on hatch time. Similarly, Vasquez and Bohren (1978) reported that selection for fast hatch time resulted in a higher embryo weight compared with control and slow hatch time groups. Correlation between embryo weight and egg weight varies from -.17 at 8 days of incubation to .90 at hatch, with a significant effect due to strain (Hassan and Nordskog, 1971). However, no evidence of correlation between egg weight and hatch time was obtained by Smith and Bohren (1974).

The present study was designed to determine if there are differences due to genotype and age of the breeder on chick weight, weight loss, hatch time, and mortality of broiler breeder eggs, as well as the correlation between these characteristics. Further, the effects of breeder genotype and age on egg components and yolk to albumen ratio were evaluated.

Materials and Methods

Experiment 1

The objective of this experiment was to explore the effects of strain and age of the female breeder on chick weight and hatch time. Three thousand three hundred eggs from 5 broiler breeder strains (A, B, C, D, and E) at three different ages (29, 47 and 57 wk) were obtained from a broiler breeder company (Arbor Acres)[1], divided into groups of 30 eggs, individually identified, weighed to the nearest .1 gram and placed in setting trays to be incubated. Two Jamesway 252 incubators were used and 11 replicates by strain were randomly distributed in each incubator. The average temperatures were 37.4, 37.2 dry bulb, and 28.5 and 28.4 wet bulb in incubators 1 and 2. The time the incubators were turned on was recorded as the hour zero of the experiment. At 456 hours of incubation, the eggs were candled to determine infertile eggs as well as dead embryos. All eggs were weighed and those containing viable embryos were transferred to hatching baskets and returned to the incubator. At 480 hours of incubation, and every 2 hours thereafter, the incubators were opened, the hatched chicks were weighed. Chick weight and hatch time were recorded. The response variables considered in this experiment were egg weight at set (set weight) and transfer (transfer weight), chick weight at hatch (hatch weight) and hatch time. Mortality was not recorded because the unhatched eggs were inadvertently destroyed; thus fertility was not verified and only hatchability of the total eggs set was calculated (total hatchability).

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[1] Arbor Acres Farm, Inc., Glastonbury, CT 06033.

Experiment 2

The length of hatch time, chick weight and weight loss, as well as the relative value of egg components as affected by age and genotype of the breeders were tested in this experiment. Two hundred eggs from each of 12 genetic crosses (six females lines , each crossed with 2 different male lines and identified as 1A, 1B, 2A, 2B, 3A, 3C, 4A, 4D, 5A, 5E, 6A, and 6F) at three different ages (29, 41, and 52 wk) were obtained from the same broiler breeder company as for experiment 1 and divided into two groups. A group of 150 eggs was randomly distributed in smaller groups of 15 eggs and allocated to each of 10 replicates per strain. Then, the eggs were individually identified, weighed to nearest .1 gram and placed in setting trays to be incubated. The time the incubator[2] was turned on was recorded as the hour zero of the experiment. At 456 hours of incubation, the eggs were candled to determine infertile eggs as well as dead embryos. Eggs containing viable embryos were weighed, transferred to hatching baskets provided with dividers to individually identify the chicks, and then returned to the incubator. At 480 hours of incubation and every 2 hours thereafter, the incubator was opened, the hatched chicks were weighed and hatch time was recorded. The chicks were returned to the incubator and held until process time. At this time each chick was weighed and removed from the incubator. The process time was at 512 hours of incubation.

All unhatched eggs were broken out and examined macroscopically for characterization of embryonic development. Eggs were classified as infertile and dead.

The response variables considered in this experiment were egg weight at set and transfer (set weight, transfer weight), chick weight at hatch and process (hatch weight, process weight), weight loss, hatch time (recorded in hours) and mortality. Weight loss was defined as weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, and weight loss total = set - process. Process weight was considered as the weight of the chick when it was removed from the incubator.

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[2] Jamesway 252

Another group of eggs from each female line was used to determine egg components. Eggs individually identified were weighed to the nearest .1 gram and hardboiled (Lee, 1985) and then cooled for 5 minutes in cold running water. The boiled egg was reweighed, shell, yolk and albumen were separated and their weights recorded. Weight loss of the eggs after boiling was in the range of 1.5 to 2% for all the strains and did not significantly change the relative values of the components.

Statistical Analysis

All the effects were considered fixed in both experiments; therefore the experimental errors were used as error terms in the analyses. The data of experiment 1 were analyzed as a 5 x 3 x 2 factorial with strain, age and incubator (INC) as main effects. A preliminary analysis of the data indicated no significant effect of the three factors interaction on any of the variables considered; therefore this interaction was not considered in the final model. The data for weight and weight loss in experiment 2 were analyzed as a 12 x 3 factorial with cross and age as main effects.

To reduce the variability due to egg weight, a least squares analysis of variance was performed using set weight as a covariable in all the analyses. Least squares means comparisons were done by orthogonal contrasts. Comparison among males indicated only a significant effect of male D v F for weight loss 3 (P = .039). Because least squares means values of the crosses 4D and 6F for this variable were not very different, the values for each female strain were pooled not considering males in the model and reducing the number of strains to six. Egg components expressed as percentage of egg weight were transformed to natural logarithms prior to the statistical analysis, but this did not alter interpretation of the results; therefore untransformed percentages analyses and least squares means percentages are presented in tables. The analyses were performed using the General Linear Model (GLM) and Correlation procedures of SAS (SAS Institute, 1989). Partial correlations coefficients were obtained by a multivariate analysis of variance (MANOVA) and data concerning total hatchability (experiment 1) and mortality (experiment 2) were analyzed using the SAS Categorical Model (CATMOD) procedure. Comparisons of least squares means for set weight and adjusted least squares means for the other variables were done by orthogonal contrasts (Steel and Torrie, 1980). When interaction effects were significant, comparisons of strain within age were performed.

Results and Discussion

Set Weight

The effects of strain and age on set weight were statistically significant, as well as their interactions in both experiments 1 and 2. Results are reported in tables 3-1 through 3-5.

In experiment 1 (Tables 3-1 through 3-3), eggs from strain A were the heaviest and significantly different from eggs of strain D and E, but not from strain B. Eggs from strain D were significantly different from strain E. All the strains were significantly different from strain C, which had the lightest eggs. Set weight increased significantly with age of the female (Table 3-2). Least squares means comparisons within age indicated that eggs from strain B were the heaviest at 29 and 57 weeks of age (Table 3-2), but they were the lightest at

47 weeks of age. The opposite was true for strain C, which laid the lightest eggs at 29 and 57 weeks of age. All comparisons within age were significantly different, except for A, B v D, E at 47 and 57 weeks of age. Although the analysis of variance indicated no significant effect of INC and their interactions with strain and age on set weight, some comparisons within incubator were statistically significant in both incubators (Table 3-1). These significances were considered not to be biologically important because set weight was measured at the beginning of the experiment.

In experiment 2, the effects of strain and age on set weight were similar to those obtained in experiment 1. Highly significant effects ($P < .001$) were observed for all the variables, except for weight loss 3 and hatch time when set weight was not considered as covariable (data not shown). Probability values for weight loss 3 were .027 for strain, .011 for age and .052 for the interaction. Hatch time was affected by age ($P = .001$), but not by strain ($P = .096$) and the interaction strain x age ($P = .792$).

Transfer Weight

Transfer weight adjusted by set weight was significantly affected by the main effects and their interactions in experiment 1 (Tables 3-1 through 3-3). Strain D had the lowest transfer weight. Comparisons of least squares means indicated that strains A and B and D and E performed similarly. Similar effects were observed between A and B within age 47 and 57, but not within age 29. Transfer weight increased linearly with age of the female in experiment 1 (Tables 3-1 and 3-2), but not in experiment 2 (Table 3-5), although the quadratic response was highly significant in experiments 1 and 2 (Tables 3-1 and 3-4).

The INC effects on transfer weight were significant for strain A v B in incubator 1, and strains A and B v D and E in incubator 2. It may be due to different responses among the strains due to humidity, because wet bulb temperature was the major observed difference between the two incubators used in experiment 1.

Hatch Weight

Hatch weight, as the other variables, was significantly affected by the factors involved in experiment 1. It was lowest for strain C, which was significantly different from all other strains (Table 3-1). All the least squares means comparison within age were significantly different, except for strains D v E within age 29, strain C v all others and A and B v D and E within age 47 in experiment 1 (Table 3-2). In experiment 2, hatch weight was not affected by the genotype of the hens (Tables 3-3 and 3-4). Hatch weight showed a significant curvilinear effect due to age of the hen in experiment 1 (Tables 3-1 and 3-2).

Process Weight

Process weight (experiment 2) was affected by age of the breeders. However, comparisons within age no indicated significant differences (Tables 3-4 and 3-5). Linear and quadratic effects of age were significant on this variable.

Weight Loss

Effects on weight loss were not consistent. Weight loss 2 was affected only by the curvilinear effect of age (Table 3-3). Weight loss 3 was significantly different for strains 1 v 2 and 5 v 6 within age 41 (Table 3-4).

Table 3-1. Probability values from least squares analysis of variance and orthogonal contrasts for characteristics of broiler breeder eggs affected by strain and age of female breeders, Experiment 1

Effect	d.f.	Set weight[1]	Transfer weight	Hatch weight	Hatch time
Strain	4	.001	.034	.001	.001
Age	2	.000[2]	.001	.000	.001
Strain x Age	8	.001	.001	.001	.001
Incubator (INC)	1	.367	.221	.107	.001
Strain x INC	4	.273	.193	.177	.018
Age x INC	2	.157	.172	.091	.010
Set weight	1	...	.000	.001	.018
Error term	7594				
Orthogonal contrasts					
Strain C v all others	1	.001	.032	.004	.005
Strain A,B v D,E	1	.001	.317	.001	.001
Strain A v B	1	.215	.447	.146	.481
Strain D v E	1	.001	.114	.001	.307
Age linear	1	.001	.001	.000	.082
Age quadratic	1	.035	.361	.001	.001
Age within INC					
Age linear, within INC 1	1	...	.020	.001	.001
Age quadratic, within INC 1	1	...	.013	.001	.001
Age linear, within INC 2	1	...	.127	.001	.001
Age quadratic, within INC 2	1	...	.207	.001	.001

[1] Set weight used as covariable for other responses.

[2] $P < .001$.

Table 3-1--continued

Effect	d.f.	Set weight[1]	Transfer weight	Hatch weight	Hatch time
Strain within age 29					
Strain C v all others	1	.001	.244	.001	.064
Strain A,B, v D,E	1	.001	.174	.001	.001
Strain A v B	1	.001	.178	.005	.039
Strain D v E	1	.030	.004	.468	.024
Strain within age 47					
Strain C v all others	1	.000	.008	.410	.246
Strain A,B v D,E	1	.652	.471	.096	.002
Strain A v B	1	.001	.217	.001	.001
Strain D v E	1	.001	.170	.001	.045
Strain within age 57					
Strain C v all others	1	.001	.002	.001	001
Strain A,B v D,E	1	.712	.002	.001	.004
Strain A v B	1	.001	.267	.001	.007
Strain D v E	1	.001	.209	.001	.042
Strain within incubator 1					
Strain C v all others	1	.187	.581	.001	.562
Strain A,B v D,E	1	.032	.003	.001	.012
Strain A v B	1	.512	.228	.098	.312
Strain D v E	1	.317	.676	.002	.410
Strain within incubator 2					
Strain C v all others	1	.118	.538	.001	.032
Strain A,B v D,E	1	.072	.233	.001	.001
Strain A v B	1	.532	.681	.490	.784
Strain D v E	1	.287	.021	.004	.430

[1] Set weight used as covariable for other responses.

Table 3-2. Least squares means for characteristics of broiler breeder eggs by strain and age of female breeders, Experiment 1

Effect	Set weight (g)[1]	Transfer weight (g)	Hatch weight (g)	Hatch time (hour)
Strain				
A	62.92	55.37	46.58	498.5
B	62.74	55.33	46.41	498.8
C	60.66	55.31	45.41	497.0
D	62.27	55.20	46.18	496.9
E	60.72	55.29	45.62	496.6
Age (weeks)				
29	56.70	55.00	42.85	498.6
47	61.99	55.40	46.42	494.8
57	66.88	55.49	48.84	499.4
Incubator				
1	61.82	55.34	46.19	498.8
2	61.90	55.26	45.89	496.3

[1] Set weight (61.50 g) used to adjust other responses

Table 3-2--continued

Effect	Set weight (g)[1]	Transfer weight (g)	Hatch weight(g)	Hatch time (hour)
Strain x Age				
A - 29	57.24	55.09	43.09	499.0
B - 29	58.63	54.97	43.65	500.2
C - 29	55.47	55.07	42.36	497.9
D - 29	56.34	54.81	42.54	498.3
E - 29	55.84	55.07	42.63	497.5
A - 47	64.06	55.33	47.42	496.7
B - 47	59.73	55.41	45.14	494.0
C - 47	62.38	55.55	46.52	495.1
D - 47	62.71	55.41	46.93	493.6
E - 47	61.07	55.31	46.09	494.6
A - 57	67.45	55.69	49.23	500.0
B - 57	69.86	55.61	50.43	502.2
C - 57	64.11	55.31	47.34	497.9
D - 57	67.76	55.38	49.06	498.9
E - 57	65.24	55.48	48.12	497.7

[1] Set weight (61.50 g) used to adjust other responses.

Table 3-2--continued

Effect	Set weight (g)[1]	Transfer weight (g)	Hatch weight (g)	Hatch time (hour)
Strain x INC				
A - 1	63.11	55.46	46.81	499.4
B - 1	62.63	55.36	46.53	499.9
C - 1	60.55	55.36	45.55	498.3
D - 1	62.27	55.25	46.35	498.5
E - 1	60.53	55.25	45.70	498.1
A - 2	62.72	55.28	46.34	497.6
B - 2	62.85	55.29	46.28	497.6
C - 2	60.76	55.25	45.27	495.6
D - 2	62.27	55.15	46.01	495.4
E - 2	60.90	55.32	45.53	495.1
Age x INC				
29 - 1	56.70	54.95	43.17	497.2
47 - 1	62.04	55.57	46.58	497.1
57 - 1	66.72	55.50	48.82	502.2
29 - 2	56.71	55.05	42.54	499.9
47 - 2	61.94	55.24	46.26	492.5
57 - 2	67.05	55.49	48.86	496.5

[1] Set weight (61.50 g) used to adjust other responses.

Table 3-3. Probability values from least squares analysis of variance and orthogonal contrasts for characteristics of broiler breeder eggs affected by strain and age of female breeders, Experiment 2

		Egg weight		Chick weight		Weight loss[1]				
Effect	d.f.	Set[2]	Transfer	Hatch	Process	1	2	3	Total	Hatch time
Strain	5	.001	.343	.617	.817	.343	.216	.037	.817	.133
Age	2	.001	.193	.489	.022	.193	.067	.054	.022	.015
Strain x Age	10	.001	.651	.035	.642	.651	.743	.056	.642	.774
Set weight	1	...	.000[3]	.000	.001	.001	.001	.010	.001	.094
Error term	2670									
Orthogonal contrasts										
Strain 3,4 v all others	1	.001	.446	.124	.661	.446	.714	.397	.661	.052
Strain 1,2 v 5,6	1	.001	.092	.095	.227	.092	.281	.141	.227	.469
Strain 1 v 2	1	.001	.122	.454	.771	.122	.283	.365	.771	.861
Strain 3 v 4	1	.001	.142	.403	.739	.142	.406	.716	.739	.754
Strain 5 v 6	1	.001	.017	.158	.647	.017	.401	.184	.647	.501
Age linear	1	.000	.010	.532	.027	.010	.328	.104	.027	.001
Age quadratic	1	.001	.172	.217	.012	.172	.092	.058	.012	.001

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch -process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] $P < .001$.

Table 3-4. Probability values of orthogonal contrasts for characteristics of broiler breeder eggs affected by strain and age of female breeders, Experiment 2

Effect	d.f.	Egg weight		Chick weight		Weight loss[1]				Hatch time
		Set[2]	Transfer	Hatch	Process	1	2	3	Total	
Strain within age 29										
Strain 3,4 v all others	1	.001	.961	.611	.610	.961	.159	.187	.610	.144
Strain 1,2 v 5,6	1	.001	.161	.269	.221	.161	.229	.217	.221	.655
Strain 1 v 2	1	.001	.416	.425	.852	.416	.952	.208	.852	.650
Strain 3 v 4	1	.001	.270	.943	.739	.270	.877	.905	.739	.573
Strain 5 v 6	1	.001	.245	.943	.193	.245	.193	.177	.193	.612
Strain within age 41										
Strain 3,4 v all others	1	.365	.158	.278	.159	.158	.871	.278	.159	.394
Strain 1,2 v 5,6	1	.012	.144	.178	.229	.144	.146	.615	.229	.794
Strain 1 v 2	1	.001	.287	.095	.882	.287	.992	.040	.882	.609
Strain 3 v 4	1	.566	.287	.674	.877	.287	.719	.435	.877	.744
Strain 5 v 6	1	.512	.456	.117	.548	.456	.548	.037	.548	.543
Strain within age 57										
Strain 3,4 v all others	1	.522	.224	.139	.871	.224	.152	.116	.871	.295
Strain 1,2 v 5,6	1	.001	.137	.092	.146	.137	.169	.152	.146	.919
Strain 1 v 2	1	.001	.133	.688	.606	.133	.606	.254	.606	.295
Strain 3 v 4	1	.017	.290	.342	.719	.290	.443	.977	.719	.932
Strain 5 v 6	1	.067	.202	.072	.276	.202	.276	.252	.276	.511

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch -process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

Table 3-5. Least squares means for characteristics of broiler breeder eggs by strain and age of female breeders, Experiment 2

	Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				Hatch time (hour)
Effect	Set[2]	Transfer	Hatch	Process	1	2	3	Total	
Strain									
1	64.95	58.68	48.34	45.83	5.93	10.04	2.53	18.48	501.7
2	67.01	58.44	48.26	45.87	6.17	9.84	2.40	18.44	501.9
3	63.40	58.84	48.65	45.96	5.77	9.90	2.68	18.34	499.5
4	65.01	58.68	48.57	45.92	5.93	9.80	2.64	18.38	499.3
5	64.16	58.61	48.14	45.99	6.00	9.75	2.58	18.32	500.8
6	63.25	58.94	48.80	45.93	5.67	9.85	2.87	18.37	501.2
Age (weeks)									
29	58.26	59.05	48.62	45.94	5.56	10.14	2.67	18.37	510.5
41	66.04	58.39	48.28	45.58	6.22	9.77	2.70	18.73	495.1
52	69.59	58.66	48.69	46.23	5.92	9.68	2.48	18.07	496.8

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight (64.61 g) used to adjust other responses.

Table 3-5--continued

Effect	Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				Hatch time (hour)
	Set[2]	Transfer	Hatch	Process	1	2	3	Total	
Strain x Age									
1 - 29	58.66	58.99	48.46	46.11	5.62	10.30	2.27	18.19	512.6
2 - 29	60.47	58.82	48.60	46.10	5.73	10.04	2.52	18.20	511.6
3 - 29	55.23	59.12	48.59	45.80	5.49	10.23	2.79	18.51	510.1
4 - 29	58.86	58.97	48.58	45.77	5.64	10.06	2.81	18.54	508.9
5 - 29	59.17	59.03	48.76	46.06	5.58	10.02	2.69	18.24	510.3
6 - 29	57.19	59.30	48.74	45.79	5.31	10.17	2.94	18.51	509.5
1 - 41	66.29	58.44	48.27	45.47	6.17	9.84	2.80	18.35	495.8
2 - 41	68.08	58.04	47.89	45.48	6.57	9.73	2.41	18.83	494.7
3 - 41	66.09	58.54	48.44	45.53	6.07	9.84	2.91	18.78	494.04

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight (64.61 g) used to adjust other responses.

Table 3-5--continued

	Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				Hatch time (hour)
Effect	Set[2]	Transfer	Hatch	Process	1	2	3	Total	
Strain x Age									
4 - 41	65.98	58.45	48.37	45.60	6.16	9.70	2.81	18.71	494.7
5 - 41	64.87	58.31	48.24	45.75	6.30	9.76	2.48	18.55	495.0
6 - 41	64.95	58.54	48.50	45.63	6.07	9.76	2.87	18.68	496.3
1 - 52	69.90	58.60	48.30	45.90	6.01	9.97	2.52	18.40	496.8
2 - 52	72.47	58.40	48.30	46.02	6.21	9.75	2.27	18.28	499.4
3 - 52	68.89	58.86	48.92	46.56	5.75	9.64	2.35	17.75	494.4
4 - 52	70.20	58.63	48.76	46.40	5.98	9.61	2.36	17.91	494.2
5 - 52	68.45	58.49	48.71	46.14	6.12	9.48	2.57	18.16	497.2
6 - 52	67.60	58.98	49.16	46.37	5.63	9.61	2.79	17.94	498.6

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight (64.61 g) used to adjust other responses.

Weight loss total was affected only by age of the breeders.

Hatch Time

Hatch time was affected by all factors involved in experiment 1 (Tables 3-1 and 3-2), but not in experiment 2 (Tables 3-3 through 3-5), in which the only significant effect was age of the breeder. In both experiments, hatch time showed a significant curvilinear response with the lowest value at the middle age of the breeder (47 and 41 weeks for experiment 1 and 2).

Pearson and partial correlation coefficients between the variables studied in experiment 2 are given in table 3-6. Correlations between hatch time and the other variables were significant and negative. Partial correlation coefficients between hatch time and the other variables were lower and nonsignificant than Pearson correlation coefficients.

Total Hatchability and Embryonic Mortality

There were significant effects of strain and age, as well of the interaction strain x age, on total hatchability in experiment 1. A significant curvilinear response associated with the age of the female breeder was observed (Table 3-7). It seems that strains C and E, which had the lowest set and hatch weight (Table 3-2), had the best hatchability, but this relationship was not evident when interaction effects were involved (Table 3-8).

Embryonic mortality in experiment 2 was mainly affected by age, with significant effects due to strain and the interaction strain x age when strain 2 was involved, which was significantly different from the others (Tables 3-9 and

Table 3-6. Pearson and partial correlation coefficients between characteristics of broiler breeder eggs during incubation, Experiment 2

	Set weight	Transfer weight	Hatch weight	Process weight	Hatch time	Weight loss[1]			
						1	2	3	Total
Set weight	**1.000**	.981[2]	.969	.953	-.226	.349	.613	.062	.655
Transfer weight	.965[3]	**1.000**	.979	.958	-.213	.161	.644	.084	.594
Hatch weight	.934	.958	**1.000**	.964	-.228	.232	.477	.131	.541
Process weight	.895	.906	.918	**1.000**	-.144	.259	.539	-.136	.398
Hatch time	.038	.036	.015	.140	**1.000**	-.133	-.065	-.308	-.324
Weight loss 1	.152	-.109	-.067	-.019	.007	**1.000**	.017	-.096	.508
Weight loss 2	.522	.569	.310	.371	.075	-.167	**1.000**	-.137	.559
Weight loss 3	.071	.102	.175	.227	-.311	-.118	-.161	**1.000**	.532
Weight loss total	.479	.400	.306	.060	-.188	.382	.448	.604	**1.000**

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - proc, weight loss total = set - process.

[2] Pearson correlations above diagonal.

[3] Partial correlations below diagonal.

Table 3-7. Probability values from categorical analysis and orthogonal contrasts for total hatchability of broiler breeder eggs affected by strain and age of female breeders, Experiment 1

Source	d.f.	Chi-Square[1]	Probability
Intercept	1	3115.67	.000[2]
Strain	4	80.88	.000
Age	2	226.64	.000
Strain x Age	8	32.07	.000
Incubator (INC)	1	19.67	.000
Strain x INC	4	4.11	.391
Age x INC	2	.16	.921
Residual	8	4.36	.399
Orthogonal contrasts			
Strain C v all others	1	12.81	.000
Strain A, B v D, E	1	.16	.685
Strain A v B	1	.26	.101
Strain D v E	1	68.30	.000
Age linear	1	174.06	.000
Age quadratic	1	75.15	.000
Strain C v all others x linear	1	1.15	.281
Strain A, B v D, E x linear	1	.08	.774
Strain A v B x linear	1	1.64	.293
Strain D v E x linear	1	21.89	.000
Strain C v all x quadratic	1	1.49	.222
Strain A, B v D, E x quadratic	1	3.94	.042
Strain A v B x quadratic	1	13.07	.000
Strain D v E x quadratic	1	.068	.409

[1] CATMOD procedure.

[2] $P < .001$.

Table 3-8. Total hatchability of broiler breeder eggs by strain and age of female breeders, Experiment 1

Effect	Eggs hatched	Eggs not hatched	Total hatchability (%)
Strain			
A	1487	493	75.10
B	1528	452	77.17
C	1577	403	79.65
D	1397	587	70.35
E	1610	370	81.31
Age (weeks)			
29	2680	620	81.21
47	2693	607	81.61
57	2222	1078	67.33

Table 3-8--continued

Effect	Eggs hatched	Eggs not hatched	Total hatchability (%)
Strain x Age			
A - 29	531	129	80.45
B - 29	535	125	81.06
C - 29	538	122	81.50
D - 29	488	172	73.94
E - 29	588	72	89.09
A - 47	519	141	78.64
B - 47	567	93	85.91
C - 47	558	102	84.55
D - 47	501	159	75.91
E - 47	548	112	83.03
A - 57	437	223	66.21
B - 57	426	234	64.55
C - 57	481	179	72.88
D - 57	404	256	61.21
E - 57	474	186	71.82

Table 3-9. Probability values from categorical analysis and orthogonal contrasts for embryonic mortality of broiler breeder eggs affected by strain and age of female breeders, Experiment 2

Source	d.f.	Chi-Square[1]	Probability
Intercept	1	3156.50	.000[2]
Strain	5	14.19	.014
Age	2	8.47	.004
Strain x Age	10	23.41	.009
Orthogonal contrasts			
Strain 2 v all others	1	12.02	.000
Strain 4 v 1,3,5,6	1	3.36	.075
Strain 1,3, v 5,6	1	.22	.642
Strain 1 v 3	1	.90	.842
Strain 5 v 6	1	.09	.942
Age linear	1	.17	.678
Age quadratic	1	3.51	.061
Strain 2 v all others x linear	1	1.16	.585
Strain 4 v 1,3,5,6 x linear	1	.76	.681
Strain 1,3 v 5,6 x linear	1	.01	.904
Strain 1 v 3 x linear	1	2.58	.286
Strain 5 v 6 x linear	1	2.25	.133
Strain 2 v all others x quadratic	1	2.51	.113
Strain 4 v 1,3,5,6 x quadratic	1	3.21	.125
Strain 1,3 v 5,6 x quadratic	1	.08	.882
Strain 1 v 3 x quadratic	1	.39	.532
Strain 5 v 6 x quadratic	1	.16	.693

[1] CATMOD procedure.

[2] $P < .001$.

Table 3-10. Embryonic mortality of broiler breeder eggs by strain and age of female breeders, Experiment 2

Effect	Total eggs	Eggs hatched	Eggs not hatched	Mortality (%)
Strain				
1	338	311	27	7.99
2	319	273	46	14.42
3	369	346	23	6.23
4	364	341	23	6.32
5	381	352	29	7.61
6	343	316	27	7.87
Age (weeks)				
29	752	677	75	9.97
41	657	619	38	5.78
52	705	343	62	8.79

Table 3-10--continued

Effect	Total eggs	Eggs hatched	Eggs not hatched	Mortality (%)
Strain x Age				
1 - 29	110	97	13	11.82
2 - 29	130	108	22	16.92
3 - 29	130	122	8	6.15
4 - 29	130	122	8	6.15
5 - 29	126	111	15	11.90
6 - 29	126	117	9	7.14
1 - 41	113	104	9	7.96
2 - 41	97	92	5	5.15
3 - 41	114	108	6	5.26
4 - 41	114	110	4	3.51
5 - 41	116	108	8	6.90
6 - 41	103	97	6	5.83
1 - 52	115	110	5	4.35
2 - 52	92	73	19	20.65
3 - 52	125	116	9	7.20
4 - 52	120	109	11	9.17
5 - 52	139	133	6	4.32
6 - 52	114	102	12	10.53

and 3-10. This strain had the highest percentage of mortality, which was characterized by a higher incidence of malposition (data not shown).

Egg Components

The analysis of variance for egg components indicated significant effects on yolk to albumen ratio (Y:A) due to genotype and age of the female breeder and their interactions (Tables 3-11 and 3-12). The yolk to albumen ratio increased linearly with age of the breeder as a consequence of a linear decrease of albumen. The comparisons of percentages of yolk within age indicated significant differences between strains 3 v 4 within age 29 and 41 weeks. These differences were consistent for albumen weight and Y:A within age 41. Within age 52, only strains 5 v 6 were significantly different in albumen weight and Y:A ratio. No significant effects were found for shell weight.

Considerable changes in characteristics of broiler chickens due to artificial selection have occurred in the last 30 years (McCarthy and Siegel, 1983). As a correlated response, egg weight has improved under selection in commercial strains (Rodda *et al.*, 1977). Selection for high and low body weight in dwarf White Leghorn resulted in a significant divergence in egg weight between lines in a study reported by Benoff and Renden (1983). The sex-linked feathering locus seems to be involved in egg size. Late-feathering dams lay heavier eggs than early-feathering dams (Lowe *et al.*, 1965; Brake, 1988; Katanbaf *et al.*, 1989; O'Sullivan *et al.*, 1991). In both experiments 1 and 2, the strains differed in initial egg weight. Causal factors that influence egg weight are yolk, albumen and shell weight (Cherry *et al.*, 1978).

Table 3-11. Probability values from least squares analysis of variance and orthogonal contrasts for egg weight and percentage of egg components Experiment 2

Effect[1]	d.f.	Egg weight	Shell weight	Yolk weight	Albumen weight	Ratio (Y:A)
Strain	5	.001	.033	.014	.001	.004
Age	2	.001	.612	.001	.001	.001
Strain x Age	10	.017	.177	.132	.052	.063
Error term	674					
Orthogonal contrasts						
Strain 3,4 v all others	1	.001	.805	.052	.043	.042
Strain 1,2 v 5,6	1	.001	.144	.333	.122	.244
Strain 1 v 2	1	.001	.256	.257	.293	.269
Strain 3 v 4	1	.001	.265	.002	.038	.010
Strain 5 v 6	1	.372	.639	.048	.028	.008
Age linear	1	.001	.498	.001	.001	.001
Age quadratic	1	.001	.668	.010	.463	.263

[1] As percentage of egg weight.

Table 3-11--continued

Effect	d.f.	Egg weight	Shell weight	Yolk weight	Albumen weight	Ratio (Y:A)
Strain within age 29						
Strain 3,4 v all others	1	.090	.603	.678	.277	.418
Strain 1,2 v 5,6	1	.472	.369	.331	.364	.568
Strain 1 v 2	1	.001	.164	.196	.112	.185
Strain 3 v 4	1	.062	.976	.038	.187	.086
Strain 5 v 6	1	.095	.756	.066	.010	.123
Strain within age 41						
Strain 3,4 v all others	1	.192	.138	.004	.023	.010
Strain 1,2 v 5,6	1	.010	.124	.143	.006	.032
Strain 1 v 2	1	.012	.178	.282	.155	.245
Strain 3 v 4	1	.001	.125	.001	.006	.002
Strain 5 v 6	1	.001	.783	.668	.535	.635
Strain within age 52						
Strain 3,4 v all others	1	.011	.738	.485	.679	.564
Strain 1,2 v 5,6	1	.024	.761	.411	.872	.646
Strain 1 v 2	1	.001	.888	.168	.185	.186
Strain 3 v 4	1	.255	.119	.317	.818	.491
Strain 5 v 6	1	.001	.350	.102	.005	.044

[1] As percentage of egg weight.

Table 3-12. Least squares means for egg weight and percentage of egg components by strain and age of female breeders, Experiment 2

Effect	No eggs	Egg weight (g)	Shell weight (%)[1]	Yolk weight (%)[1]	Albumen weight (%)[1]	Ratio (Y:A)
Strain						
1	109	64.05	10.19	30.42	57.22	.534
2	111	66.79	9.94	29.78	58.76	.508
3	119	62.08	9.92	29.99	58.22	.516
4	112	65.19	10.05	29.60	58.52	.508
5	116	64.03	9.87	30.60	57.79	.548
6	126	63.71	10.05	30.59	57.23	.537
Age (weeks)						
29	120	57.73	9.97	27.22	60.14	.458
41	318	65.58	9.98	30.60	57.84	.530
52	255	69.62	10.05	32.71	55.93	.587

[1] As percentage of egg weight.

Table 3-12--continued

Effect	No eggs	Egg weight (g)	Shell weight (%)[1]	Yolk weight (%)[1]	Albumen weight (%)[1]	Ratio (Y:A)
Strain x Age						
1 - 29	20	56.77	10.24	27.63	58.98	.466
2 - 29	20	60.15	9.88	26.19	61.43	.421
3 - 29	20	55.77	9.87	27.37	60.16	.451
4 - 29	20	58.01	9.92	26.42	60.97	.430
5 - 29	20	58.95	9.98	28.30	59.47	.525
6 - 29	20	56.75	9.96	27.39	59.82	.457
1 - 41	46	65.28	10.28	30.80	57.37	.538
2 - 41	54	67.14	9.70	30.60	58.83	.525
3 - 41	55	61.89	10.14	30.45	58.24	.523
4 - 41	53	68.47	9.97	29.66	58.57	.508
5 - 41	54	65.91	9.70	30.60	58.37	.525
6 - 41	56	64.76	10.06	31.55	56.25	.562
1 - 52	43	70.10	10.06	33.04	55.32	.598
2 - 52	37	73.10	10.12	32.84	55.61	.592
3 - 52	45	68.58	10.14	32.15	56.26	.574
4 - 52	37	69.10	9.88	32.72	56.01	.586
5 - 52	43	67.22	10.08	32.90	55.51	.594
6 - 52	50	69.62	10.02	32.59	56.67	.577

[1] As percentage of egg weight.

A high genetic correlation (r = .78 and .91) exists between both yolk and albumen weight and egg weight (Rodda *et al.*, 1977). Differences in egg weight among various lines of chickens have been attributed to differences in yolk, albumen, and shell weight (Marion *et al.*, 1964; Cherry *et al.*, 1978; Benoff and Renden, 1983). The weight of the albumen has the largest effect on egg weight (Rodda *et al.*, 1977; Benoff and Renden, 1983). Results obtained in the present study in relation to differences in egg weight, egg components and Y:A ratio agreed with previous results for strain 2 and 4, but not for the others. Strains 2 and 4 had the highest egg weight and albumen weight (Tables 3-5 and 3-12). Strain 3 had the lowest egg weight; however, albumen weight was not the lowest one, while strain 1, which had the lowest albumen weight, was intermediate in relation to egg weight (Table 3-12). This may indicated differences other than egg weight among the strains studied. The albumen influence on egg weight decreases as body weight increases due to a reduction of the magnum weight per unit surface area (Benoff and Renden, 1983). Furthermore, the oviduct transition time and body weight of the breeder may affect egg weight and egg components. Smaller bodied birds seem to use more of their body resources for reproduction than larger bodied bird (Benoff and Renden, 1983), and the largest body size strains do not necessarily lay the largest eggs (Bray and Iton, 1962); although Kinney (1969) indicated phenotypic and genetic correlations higher than .70 between body weight and egg weight. Significant strain differences in egg components and yolk percentage have been found (Arafa *et al.*, 1982; Carey, 1988). Anatomical and physiological differences, beside differences in feathering locus, or body weight among the strains considered, could explain some discrepancies and significant genotype x environmental interactions observed for some strains in the present study. However the existence of these differences is unknown.

Age of the breeder affects egg weight (Fletcher *et al.*, 1981, 1983; Reinhart and Hurnik, 1984) and consequently egg components and their ratio (Cunningham *et al.*, 1960; Anthony *et al.*, 1989a; O'Sullivan *et al.*, 1991; Hussein *et al.*, 1993). Percent yolk progressively increases while percent albumen decreases in older hens. Shell weight seems to decrease by age of the hen (Marion *et al.*, 1964; Fletcher *et al.*, 1981; Arafa *et al.*, 1982). Fletcher *et al.* (1981) suggested it might be due to a greater increase in total egg mass as compared to the increase in shell weight. When shell weight is expressed as a percent, it is a negative function of egg weight (Fletcher *et al.*, 1983). Most of the differences in yolk and albumen weight among strains may be due to differences in egg weight, whereas differences in shell weight may be due to genetic effects (Marion *et al.*, 1964). Results obtained in the present study were consistent with those reported in the literature. Egg weight varied with age of the hen. This variation might account for variation in egg components for strains 2 and 4. However, this relationship was not found for the other strains studied, for which variation in egg components was not proportional to variation in egg weight due to age. This could be a result of different genetic backgrounds of the animals tested. Recently, Hussein *et al.* (1993) concluded that differences in yolk to albumen ratios exist between commercial layer and broiler breeder eggs. A significant curvilinear effect of age on yolk weight, but not for on the other components was observed in the present study, which is consistent with results reported by Marion *et al.* (1964) related to yolk plus albumen. Although a highly significant interaction strain x age was observed for egg weight in experiment 2, this effect was not significant for yolk and shell weight, and the probability values for albumen weight and Y:A ratio were higher than expected. Comparison of strains within age indicated that strains 5 and 6 performed differently in each age studied, which indicated a significant genotype x environment interaction effect that was not evident in the other strains.

Egg weight is a temporary environmental influence which may mask the true genetic differences in embryonic growth among strains, principally when embryo growth is measured at hatching time when a high correlation ($r = .98$) exists between egg weight and chick weight (Bray and Iton, 1962). This is consistent with the high correlation between egg and chick weight reported in the literature (reviewed by Wilson, 1991b) and the Pearson and partial correlation coefficients obtained in the present study.

Analysis using egg weight (set weight) as a covariable indicated no significant effects of strain and age on transfer and chick weight in experiment 2, although strain effects were significant for weight loss 3 and the interaction strain x age was significant for hatch and process weights. Similar results were reported by Sherman and Shultz (1989) who compared two strain crosses of domestic turkeys after removing the variation due to egg weight. The opposite was true in experiment 1, in which strain, age and the interaction strain x age were highly significant for all variables (Table 3-1). This indicated that the strains studied in experiment 1 differed from each other (except strains A and B), regardless of their egg size. Bray and Iton (1962) indicated that strains were a significant source of variation for embryo weight at different days of incubation but not at hatch when embryo weight was considered as percentage of egg weight. They suggested differences within strains in egg weight and chick weight relationship. On the contrary, Hassan and Nordskog (1971) indicated significant differences due to lines and crosses in hatch weight using egg weight as a covariable.

Tullet and Burton (1982) stated that variation in chick weight at hatch is affected by egg weight loss during incubation and the weight of shell and other residues at hatch. Weight loss from the egg is due to the loss of water and is affected by egg shell characteristics and genetic effects (Landauer, 1967; Rahn *et al.*, 1981; Arad and Marder, 1982; Christensen and McCorkle., 1982; Burton and Tullet, 1983). Furthermore, shell characteristics, which decline in aging hens, have been associated with hatchability. McDaniel *et al.* (1979) indicated a positive relationship between shell specific gravity and hatchability in eggs of young birds (36 and 52 weeks of age), but not in eggs of older birds (64 weeks of age), which had the same specific gravity, but a lower hatchability compared with the younger birds. Similar results were found by Peebles and Brake (1987) in broiler eggs. However, Brunson and Godfrey (1953) found that specific gravity in turkey eggs was not related to hatchability in most cases. The effect of age on hatchability may be partly mediated through changes in shell characteristics with increasing egg size (Hamilton, 1978). Buss (1982) indicated differences among families, strains, and breeds of chicken in shell formation. He concluded that egg shell quality is independent of egg production or egg weight. Lewis and Perry (1987) reported significant genotype effects in both shell weight and shell density. Brake (1988) indicated significantly increased egg weight and significantly decreased percent shell for slow feathering hens when compared with fast feathering hens. However, Cahaner *et al.* (1986) did not find differences in percentage of egg shell between two lines selected for abdominal fat. Results observed for hatchability for experiment 1 did not show a relationship between egg weight and hatchability in the strains studied (Tables 3-2 and 3-8), although a highly significant curvilinear effect due to age was found. Analysis of variance of embryonic mortality in experiment 2 showed a highly significant effect due to strain, age, and their interactions. However, orthogonal comparisons indicated that only strain 2 was highly significant different from all the others, with a high incidence of malpositioned embryos.

Differences in shell characteristic may explain differences in total hatchability found in experiment 1 and weight loss in experiment 2. However, no experimental evidence exists for differences in shell properties among strains in the current study. Thus, it can only be concluded that strain differences exist in weight loss and hatchability that may be affected by the age of the breeder, as indicated by the highly significant strain x age interaction and the comparison of strains within age.

Hatch time is affected by many factors (reviewed by Wilson, 1991b) and can be changed through artificial selection as a direct or correlated response. Crittenden and Bohren (1961) indicated a heritability for hatch time of .49 in agreement with Siegel *et al.* (1968), who reported heritability of .50. However, conflicting results are found in the literature relative to correlated response. A genetic correlation of .55 and .20 (from sire and dam components of variance) between hatch time and egg weight were reported by Crittenden and Bohren (1961), whereas Siegel *et al.* (1968) indicated a genetic correlation of .11 and a negative phenotypic correlation between body weight and hatch time. On the contrary, Smith and Bohren (1974) indicated no evidences of genetic correlation between hatch time and egg weight and suggested that correlations reported by Crittenden and Bohren (1961) might be considered not significant due to the magnitude of the standard errors (Smith and Bohren, 1974; Vasquez and Bohren, 1978). The former indicated that a fast-hatching line had significantly smaller egg weight than slow-hatching line and the control, but these differences were attributed to random drift and not a response correlated to selection for hatch time. A similar response related to egg weight and hatch time was observed in the present study when partial correlation coefficients were estimated (Table 3-6). Residual partial correlation between hatch time and egg weight was very low (r = .038) and non-significant. Pearson correlation coefficient between this two variables was larger and negative (r = -.226), but was not significant, in agreement with results reported by Hager and Beane (1983).

Smith and Bohren (1974, 1975) indicated no relationship between hatch time and hatchability. This agrees with results obtained in experiment 1 in the current study. However, the lowest mortality was associated with the shortest hatch time in experiment 2. This is contrary to results reported by Bohren *et al.* (1977) and Bohren (1978), who indicated that slow hatching lines of White Leghorn hens had significantly reduced hatchability under low conditions of humidity.

Results obtained in the present study indicated significant differences among the strains studied. These differences are most evident in egg weight and are sensible to be modified by environmental factors. Differences in chick weight and weight losses, as well as in egg components not related to egg weight indicated differences of the strains due to their genotype. Hatch time is not correlated to egg weight. Differences in hatch time among strains seems to be related to differences in the genetic background of the embryos, or to genotype x environment interactions effects.

CHAPTER 4
LOW TEMPERATURE EFFECTS ON EMBRYONIC DEVELOPMENT AND HATCH TIME

Introduction

One of the most important factors affecting embryonic development during the incubation period is temperature. This affects growth of the embryo, egg weight loss and duration of the incubation period in different manners. The effect of temperature on embryonic development is dependent on the temperature itself (low or high temperature), age of the embryo, duration of exposure, and interaction effects as well as humidity and type of incubator (Wilson, 1991a). A large body of information is available pertinent to the effects of temperature on hatchability including those of Barott (1937), Kosin (1964), Landauer (1967), Lundy (1969), and Wilson (1991b). The hypothermic effect on developing embryos is described in terms of physiological zero temperature (Webb, 1987). When the environmental temperature is between the normal incubation temperature, and the physiological zero (approximately from 27 to 35 C) a disproportionate development usually occurs, which suggests that tissues may have different physiological zeros; thus resulting in asynchronous growth (Wilson, 1991b).

Few studies have been done on the influence of low temperature on weight loss of the embryo and length of the incubation period. Sarpong and

Reinhart (1985) indicated no significant difference in body weight of 1 day-old cooled chicks and the control, although there was a tendency towards higher body weight of the control. As a general rule, it has been found that hatching time is delayed approximately the same as the time of cooling used. Taylor *et al.* (1933) indicated that chilling eggs by interruption of the incubator power supply for 12 hours resulted in a hatching period postponed 18 to 20 hours behind the control group. However this was not true for eggs chilled on the 21 day of incubation. Similar results related to delayed hatching time were reported by Booth and Rahn (1991), who indicated that cooling eggs for a total of 126 hours (6 hours every day) at 24 C delayed hatching time 170.4 hours. Decuypere and Michels (1992) reported a delay of only 24 hours in hatching time of eggs cooled for 54 hours (6 hours a day from day 11 to 20 of incubation).

Reduction in hatchability has been reported due to long or short-term deviations from the normal artificial incubation temperature (37.5 C). It is because of the inability of avian embryos to regulate their temperature (Oppenheim and Levin, 1975). According to Booth and Rahn (1991), one of the effects of cooling eggs during incubation was a greater mortality, and consequently, a lower hatchability. Similar results were reported by Taylor *et al.* (1933), Lancaster and Jones (1988), Decuypere and Michels (1992) and others. Sarpong and Reinhart (1985) indicated a significantly high hatchability of broiler eggs cooled for 24 hours at 16 days of incubation. The same results were obtained by Callebaut (1990) by consecutive daily 8 hours interruptions of incubation of quail eggs, but when the period of cooling was extended to 12 hours the hatchability decreased about 75%.

The discrepancies found in the literature about how much hatch time is delayed due to a specific period of cooling during incubation and its effects, suggest that interactions between the age of the embryo when the cold stress is applied, hours of exposure, and temperature at which the embryo is exposed, as well as its genotype, play important roles in this matter.

Five experiments were conducted to determine: 1) how much hatch time, as affected by cold stress, is correlated with the other variables (total and partial), 2) how much hatch time, chick weight and egg weight loss are affected by exposure of the embryo to cold stress at different ages, different temperatures, different length of exposure and their interactions during the incubation period, and 3) how much embryonic mortality is affected by the factors involved.

Materials and Methods

Eggs obtained from a broiler breeder (Arbor Acres)[1] and White Leghorn (Hy-Line W-36) breeder[2] flocks were randomly distributed in groups of 20 eggs, individually identified, weighed to nearest .1 gram and placed in setting trays to be incubated. The time the incubator[3] was turned on was recorded as the hour zero of the experiment. During the incubation period, the embryos were cooled at different ages and different hours of exposure (HS). In experiment 1 two different temperatures (TEM) were used (18 and 24 C). In the other experiments all the embryos were cooled at 24 C. This temperature was selected because it is the approximate temperature in commercial hatchery rooms and it is just below physiological zero temperature. A group was incubated under normal condition (incubator temperature of 37.5 C) through all the incubation period in each experiment and was used as control group.

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[1] Arbor Acres Farm, Inc, Glastonbury, CT 06033.

[2] Poultry Science Department, University of Florida.

[3] Jamesway 252 or Robbins IHA.

The response variables considered in all the experiments were egg weight at set and transfer (set weight, transfer weight), chick weight at hatch and process (hatch weight, process weight), weight loss, hatch time (recorded in hours) and embryonic mortality. Weight loss was defined as weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, and weight loss total = set - process. Process weight was considered as the weight of the chick when hatch was complete and all chicks were removed from the incubator.

At the age selected to apply the cold stress, the eggs were removed from the incubator and placed in a chamber at a constant temperature, or on a table at environmental temperature (hatchery room). After the hours of exposure to lower temperature selected for each treatment, the eggs were returned to the incubator. At 456 hours of incubation, the eggs were candled to determine infertile eggs and dead embryos. All eggs were weighed and those containing viable embryos were transferred to hatching baskets provided with dividers to identify the chicks individually, and then returned to the incubator. At 480 hours of incubation, and every 4 hours thereafter, the incubator was opened, the hatched chicks were weighed and hatch time was recorded. Then, the chicks were returned to the incubator and held until process time. At process time, each chick was weighed and removed from the incubator. The process time for the control group was at 512 or 516 hours of incubation. For the groups exposed to cold stress, process time was delayed the same number of hours that each group of eggs were cooled.

After the process weight was recorded and the chicks removed, the unhatched eggs were returned to the incubator for 24 hours to assure that all eggs were given full opportunity to hatch. Unhatched eggs were placed in a storage room and identified by treatment for further examination. All unhatched eggs were broken out (except for experiment 2) and examined macroscopically for characterization of embryonic development. Eggs were classified as infertile and dead (before and after treatment). In some cases it was not possible to identify whether the cause of mortality was due to treatment or due to other causes in those embryos that died after treatment.

Experiment 1

This experiment was designed to explore the effect of cooling the embryo below the average temperature considered as its physiological zero, at different ages and different HS (continuous) on hatch time. Broiler eggs (1140) were cooled at 18 C and 24 C on 14, 16 or 18 days of incubation. Three different HS to cold stress were used in this experiment (12, 24, or 36 hours). The procedure used to apply the cold stress was as previously described. The eggs were set in three adjacent Robbins IHA incubators. Average temperatures were 37.3, 37.1, and 37.3 C for the dry bulb and 30.6, 32.9, and 29.6 C for the wet bulb in incubators 1, 2, and 3. The treatments (20 eggs per treatment) were distributed at random in the front of each incubator. Process time was at 516 hours for the control groups and 552 hours for the cooled chicks. Process time for cooled chicks was determined as the time when the frequency of hatching chicks was one chick or none per treatment.

The data were analyzed as a 3 x 2 x 3 x 3 factorial plus a control group, considering age (14, 16, 18 days), temperature (18 and 24 C), HS (12, 24, and 36 hours) and incubator (INC) as main effects.

Experiment 2

This experiment was designed to test the upper limit of HS to cold stress that is possible to be used to delay hatch time with no detrimental effects on embryonic development. White Leghorn eggs (600) were divided into groups of 20 eggs each and set in the top, center and bottom of a Robbins IHA incubator. The treatments were not replicated within location in the INC. The embryos were cooled (continuously) at 12, 14, or 16 days of incubation for 24, 48, or 72 hours at 24 C. Process time was at 516 hours of incubation for the control group, 540 hours for the groups cooled for 24 hours, 564 hours for the groups cooled for 48 hours, and 588 hours for the groups cooled for 72 hours.

Unhatched eggs were not broken out because they were inadvertently destroyed; therefore, embryonic mortality before and after treatment was not determined. Fertility was determined by candling the eggs at transfer and total mortality was determined by difference between fertile eggs set and hatched.

The data were analyzed as a 3 x 2 x 3 factorial plus a control group with age (8, 12, 16 days), HS (24 and 48 hours) and location in the INC as main effects. The group cooled for 72 hours was not included in the statistical analysis because none of the eggs hatched.

Experiment 3

The effect of continuous cold stress on the young embryo was tested in this experiment. Broiler eggs (945) were incubated in three adjacent INC (same incubators used in experiment 1). Three replicates per treatment (15 eggs per replicate) were randomly distributed in each incubator. The embryos were cooled at 24 C at 8, 12, or 16 days of incubation for 24 or 36 hours. Process time for the control group was at 512 hours, and 536 and 548 hours for the groups cooled for 24 or 36 hours, respectively. The experimental procedure was as in experiment 1 and 2.

The data were analyzed as a 3 x 2 x 3 factorial plus a control and INC was considered as source of variation.

Experiment 4

The effect of intermittent cooling was tested in this experiment. Three hundred broiler eggs were divided into 3 groups and incubated in a Robbins IHA incubator. One group was used as control; the second group was cooled for 6 hours every other day (every 48 hours) starting on day 2. The third group was cooled for 12 hours every four days (every 96 hours) starting on day 4. Each of the latter groups was cooled for a total of 48 hours. To cool the eggs, they were placed on a table in the hatchery room. The average temperature in the room during the experimental period was 23 C. Each treatment was replicated 5 times (20 eggs per replicate). Process time was 512 hours for the control group and 560 hours for the cooled eggs.

The data were analyzed as a 3 x 5 factorial considering treatment and location in the INC as main effects.

Experiment 5

The response to continuous cold stress as affected by genotype of breeders, was tested in this non-replicated experiment. One hundred and twenty five eggs from each of 10 Arbor Acres genetic crosses (five females lines crossed with 5 different males lines identified as 1A, 1B, 2A, 2B, 3A, 3C, 4A, 4D, 5A, and 5E) were divided into 10 groups. Eight groups were cooled at 12 or 16 days of incubation for 24 or 48 hours. The other 2 groups were used as control. Two Jamesway 252 incubators were used. The average of temperature were 37.3, 36.9 C dry bulb, and 29.8, 29.1 C wet bulb in incubators 1 and 2, respectively. Incubator was considered as source of variation in the statistical analysis. Process time was at 512 hours for the control groups and 536 and 560 hours for the groups cooled for 24 and 48 hours, respectively.

The data were analyzed as a 10 x 2 x 2 x 2 factorial plus a control.

Statistical Analysis

Eggs were not classified by weight. To eliminate bias due to egg weight, a least squares analysis of variance was performed using set weight as a covariable in the analysis of the other variables, except mortality. To get the estimator of the main effects and their interactions, the data were analyzed without the control group. This was done because if the control group were included, then empty cells in the factorial structure would make it impossible to get least squares means for main effects. Adjusted least squares mean comparisons were done by orthogonal contrasts (Steel and Torrie, 1980). To compare the treatments of the factorial versus the control group, an analysis considering treatment and incubator (or location) as a source of variation was performed (Littell *et al.*, 1991). Least squares means comparisons of the control group versus other treatments were done by orthogonal contrasts.

All the effects were considered fixed. Thus the experimental errors were used as error terms. Data for weight and hatch time were analyzed using the SAS General Linear Model (GLM) and Correlation procedures (SAS Institute, 1989). Partial correlations were obtained by a multivariate analysis of variance (MANOVA) and data concerning mortality were analyzed using the SAS Categorical Model (CATMOD) procedure.

Results and Discussion

Pearson and Partial Correlations

The coefficients for total and partial correlation followed the same pattern in all the experiments and were in the range of values reported in the literature. Only coefficients estimated for experiment 1 are reported (Table 4-1). The estimated coefficients for total correlation are shown above the diagonal. Hatch time showed a highly significant correlation with all the variables recorded before it occurred (set weight, transfer weight, hatch weight, and weight losses). All correlations were positive, except for weight loss 1. The partial correlation coefficients were a little different from the total correlation coefficients, being higher or lower for some variables, but they followed the same pattern (data not shown). When these coefficients were estimated using set weight as a covariable (Table 4-1 below diagonal), the significance as well as the values and sign of the coefficients for the correlation between hatch time and the other

Table 4-1. Pearson and partial correlation coefficients between characteristics of broiler breeder eggs exposed to cold stress during incubation, Experiment 1

	Set weight	Transfer weight	Hatch weight	Process weight	Hatch time	Weight loss[1]			
						1	2	3	Total
Set weight[2]	**1.00**	.94[3]	.91	.79	.11	.19	.49	.09	.42
Transfer weight	...	**1.00**	.95	.79	.20	-.14	.53	.15	.35
Hatch weight	...	.57[4]	**1.00**	.82	.11	-.05	.25	.16	.26
Process weight	...	.18	.57	**1.00**	.01	.07	.24	-.42	-.20
Hatch time	...	-.05	-.14	-.03	**1.00**	-.26	.30	.14	.15
Weight loss 1	...	-.98	-.57	-.18	.05	**1.00**	-.10	-.18	.24
Weight loss 2	...	.22	-.67	-.25	.13	-.22	**1.00**	.06	.43
Weight loss 3	...	.10	.14	-.86	-.03	-.10	-.08	**1.00**	.79
Weight loss total	...	-.18	-.36	-.98	.03	.18	.25	.86	**1.00**

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Used as covariable for other values.

[3] Pearson correlations above diagonal.

[4] Partial correlations below diagonal.

variables were different from total correlation coefficients. They were not significant except for hatch weight and weight loss 2, which were highly significant. Hatch time was positively correlated with weight loss 2 and negatively with hatch weight.

The variation in chick weight at hatch is determined by egg weight and egg weight loss during the incubation period (Tullet and Burton, 1982). Large chicken eggs usually require a longer incubation time than small ones (McNally and Byerly, 1936; Williams *et al.*, 1951). Significant correlation between hatch time and egg weight have been reported (Wilson, 1991b). From eggs of the same weight, the variability of chick weight at hatch is influenced for the water lost from the chick and the residual yolk sac (Tullet and Burton, 1982). This might explain differences between total correlation and estimated partial correlation coefficients in this study, because the variability due to egg weight was removed using set weight as a covariable. The highly significant and positive partial correlation between hatch weight and weight loss 2 indicated that the longer hatch time, the higher weight loss from transfer to hatch.

Transfer Weight, Chick Weight, Weight Loss, and Hatch Time

The response of the chick embryo to cold stress during the incubation period depended upon the age of the embryo at which it was cooled, hours of exposure, temperature and genotype of the breeders, as well as the incubator conditions of temperature and humidity and some interactions among the factors involved. Results are shown in tables 4-2 through 4-14.

Transfer Weight

Transfer weight was significantly affected by INC (Tables 4-2, 4-8, and 4-13), location within the incubator (Table 4-5) and female breeder (Table 4-13). No significant effects were observed due to age in any of the experiments (Tables 4-2, 4-5, 4-8, and 4-13), but the linear effect (age 14 versus age 18) was significant ($P < .029$) in experiment 1 (data not shown). Comparison with the control group indicated highly significant differences between this group and the cooled eggs in experiment 2 (Table 4-7). In experiment 1 and 3, the control group was not different from the other treatments (Tables 4-4 and 4-10). Least squares mean comparisons for crosses indicated a significant genotype x environment interaction (Table 4-14); female 3 was not different from the other female lines, but female 1 was different from female 2. In all the experiments the cooled eggs were heavier than the control as a consequence of a lower weight loss from set to transfer. Sarpong and Reinhart (1985) indicated that embryos in cooled eggs had a reduced metabolic rate, and consequently a reduced heat production, which under normal conditions reached a plateau around day 16 or 17 of incubation (Vissechedijk, 1968; Decuypere, 1979). The temperature of the egg at 11 days is above incubator environment (38 C) with subsequent increases from about .2 C in the latter half of incubation to 2 C just before hatching (Tazawa and Rahn, 1987). No compensatory increase of metabolic rate is evident when the embryo is cooled prior to around day 18 of incubation (Tazawa *et al.*, 1988). The incubator effect on transfer weight may be explained by the differences in wet bulb temperatures (WB) of the incubators used. The highest transfer weight was reached by the eggs incubated at the highest WB temperature, possibly due to a reduction of the moisture gradient between the egg and the incubator (Tullet and Burton, 1982; Swann and Brake, 1990) and less weight loss (Lundy, 1969, Gildersleeve, 1984).

Hatch Weight

Hatch weight was significantly affected by TEM and the interaction age x TEM in experiment 1 (Table 4-2). The chicks exposed to 18 C were heavier than the chicks exposed to 24 C (Table 4-4). Both groups had lower weight (45.11 and 44.90 g) than the control group (45.19 g), but these differences were not significant. In experiment 2 hatch weight was affected by age of the embryo (Table 4-5). The linear effect (age 12 versus age 16) was significant and there was an inverse relationship between hatch weight and age of the embryo (data not shown). The heaviest treated chicks were those from eggs cooled at 12 days, but they weighed less than the control (Table 4-6). Higher hatch weight of chicks exposed to low temperature has been reported (Moreng and Bryant, 1954; Kuhn *et al.*, 1982) due to a lower weight loss. The opposite was true in the present study. A higher heat production per unit of time, and per unit of dry body weight from day 11 up to the end of incubation were reported for eggs incubated at 35.8 C during the first 10 days of incubation (Geers *et al.*, 1983). This higher heat production might increase the body temperature of the embryo and the temperature gradient between the embryo's body and the incubator, which led to more evaporation; thus, to a higher weight loss. Weight loss 2 was greater for cooled eggs in experiments 2, 3, and 4. If compensatory growth occurred (Decuypere, 1979; Kuhn *et al.*, 1982; Geers *et al.*, 1983;) it was not enough to compensate for differences in weight loss of the cooled embryos versus the control. Least squares mean comparisons of hatch weight by treatment indicated that the control group was significantly different from the cooled groups in experiment 2 (Table 4-7). Hours of exposure had a highly significant effect on hatch weight in experiment 5, but its effect was not significant in the other experiments. In general the control group was heavier than the other groups, except for some second and third order interactions where incubator effects were involved in experiment 1 and 5 (Tables 4-2 and 4-13). There were no differences among the crosses, except for some interactions with age and HS (Table 4-14).

Process Weight

Process weight was mainly affected by HS when the exposure was up to 48 hours, whether the exposure was continuous as in experiment 2 or intermittent as experiment 4 (Tables 4-5 and 4-11). Hours of exposure was not significant in experiment 3, although the interactions age x HS and HS x INC were highly significant (Table 4-8). Least squares mean comparisons indicated no differences between the control group and groups cooled in experiment 1 (Table 4-4). In experiment 2, the control group was highly significantly heavier than all the other groups (Tables 4-6 and 4-7). Similar results were obtained in experiment 3 (Table 4-10). Process weight was heavier for the control group in all the experiments. This was a consequence of a higher weight loss from the cooled egg from hatch to process. Evidences of a greater weight loss after hatching of eggs cooled during incubation have been indicated by Decuypere and Michels (1992). A possible explanation could be a longer holding time for cooled eggs, which may lead to a higher degree of dehydration. However, except for results of experiment 4, the differences between the least squares means for hatch time and process time (difference in hours) were similar to the control when HS was considered as source of variation (about 10 hours). This could indicate a higher susceptibility to dehydration of the cooled chicks. The performance of the crosses for process weight was different from transfer weight. Female 4 was significantly different from female 5, but female 3 was not significantly different from the others (Table 4-14).

Weight Loss

Weight loss 1 and weight loss total were affected in a similar manner than transfer and process weights in all the experiments. Weight loss 2 and weight loss 3 exhibited different pattern in each experiment and were affected mainly by age (experiments 2 and 3), HS (experiments 1 and 5) and genotype of the breeders (experiment 5). When Robbins incubators were used, the effect of incubator on weight loss 2 and weight loss 3 was highly significant as well as some interactions (Tables 4-2 and 4-8). In experiment 4 the cooled chicks lost significantly more weight from transfer to hatch (weight loss 2) than the control (Table 4-11). The control group was not different from the cooled chicks in weight loss 3. However, these groups differed from each other (Table 4-11), which is consistent with results reported by Kuhn *et al.* (1982) and might indicate differences in body mass due to age of the embryos when they were cooled (Decuypere, 1979). Weight loss total was greater for all cooled chicks and was mainly affected by HS and the interactions HS x INC, and age x TEM x HS x INC. An effect of age of the breeders was observed. In experiment 1 the breeders were younger than in the other experiments. Weight loss total was less in this experiment. The differences in weight loss total between cooled eggs and the control were greater in experiment 2, which may indicate more susceptibility to dehydration of Leghorn eggs compared with broiler eggs, but no statistical comparison was possible. Similar results were suggested by Lancaster and Jones (1988), but the opposite was concluded by Decuypere and Michels (1992) when comparing different strains.

Hatch Time

Hatch time was affected by all factors involved and some interactions in experiments 1 and 3 (Tables 4-2 and 4-8). The quadratic effect of age was significant ($P < .053$ and $P < .022$ for experiments 1 and 3), but not the linear effect. Hours of exposure had a highly significant effect on hatch time in all the experiments. The longer the exposure period, the longer the hatch time, but in general the delay was shorter than the respective exposure period to cold stress. Hatch time in incubator 3 (experiment 1) was shorter than in incubators 1 and 2. This situation was not repeated in experiment 3, although the same incubators were used. No problems were detected by examination of the records of temperature and humidity for the time the experiments were conducted. In experiment 5, crosses had a highly significant effect (Tables 4-13 and 4-14). In other experiments with the same crosses a significant cross effect on hatch time was not found (chapter 3). This could be due to differences in experimental protocol or because of genotype x environment interactions. All interactions with crosses were highly significant, except for the interaction cross x INC. Hours of exposure and their interactions were significant. Age at which

Table 4-2. Probability values from least squares analysis of variance for characteristics of broiler breeder eggs exposed to cold stress during incubation, Experiment 1

			Chick weight		Weight loss[1]				
Effect[2]	d.f.	Transfer weight	Hatch	Process	1	2	3	Total	Hatch time
Age	2	.135	.137	.216	.135	.208	.056	.216	.006
Temperature (TEM)	1	.689	.020	.539	.689	.073	.545	.539	.000[3]
Age x TEM	2	.866	.044	.833	.866	.013	.255	.833	.125
Hour of exposure (HS)	2	.074	.133	.000	.074	.002	.001	.000	.000
Age x HS	4	.409	.080	.021	.409	.073	.004	.021	.000
TEM x HS	2	.021	.205	.828	.021	.613	.186	.828	.063
Age x TEM x HS	4	.495	.172	.779	.495	.003	.152	.779	.004
Incubator (INC)	2	.000	.000	.054	.000	.000	.000	.054	.000
Age x INC	4	.295	.031	.993	.295	.083	.439	.993	.085
TEM x INC	2	.741	.783	.032	.741	.498	.004	.032	.457
Age x TEM x INC	4	.836	.119	.925	.836	.060	.156	.925	.481
HS x INC	4	.804	.022	.002	.804	.002	.000	.002	.133
Age x HS x INC	8	.690	.228	.005	.690	.219	.012	.005	.024
TEM x HS x INC	4	.144	.163	.066	.144	.050	.038	.066	.004
Age x TEM x HS x INC	8	.759	.112	.021	.759	.201	.001	.021	.345
Set weight	1	.000	.000	.000	.000	.000	.000	.000	.017
Error term	1025								

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] $P < .001$.

Table 4-3. Least squares means for characteristics of broiler breeder eggs exposed to cold stress during incubation, versus a group incubated under normal conditions, Experiment 1

			Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				Hatch time (hour)
Effect[2]			Set	Transfer	Hatch	Process	1	2	3	Total	
Age x Temperature x Hour											
14	18	12	59.43	54.60	45.24	43.21	4.70	9.43	2.00	16.20	510.9
14	18	24	58.94	54.44	45.07	41.86	4.87	9.62	3.21	17.55	519.5
14	18	36	58.98	54.84	45.10	42.23	4.47	10.02	2.84	17.17	538.0
14	24	12	58.76	54.59	45.22	43.10	4.71	9.51	2.10	16.31	509.5
14	24	24	59.29	54.67	44.93	41.78	4.63	9.83	3.12	17.62	521.5
14	24	36	60.09	54.78	44.87	41.75	4.53	9.97	3.09	17.66	533.4
16	18	12	59.30	54.52	44.95	42.51	4.78	9.85	2.58	16.90	511.8
16	18	24	59.05	54.52	44.92	42.49	4.78	9.76	2.39	16.92	527.7
16	18	36	59.62	54.78	44.73	41.87	4.52	10.16	2.81	17.53	532.9

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Adjusted for set weight (59.25 g).

Table 4-3--continued

Effect[2]			Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				Hatch time (hour)
			Set	Transfer	Hatch	Process	1	2	3	Total	
Age x Temperature x Hour											
16	24	12	58.93	54.46	45.12	42.37	4.85	9.39	2.72	17.04	509.3
16	24	24	59.32	54.81	44.77	42.38	4.50	10.08	2.55	17.03	519.9
16	24	36	59.25	54.51	44.80	41.95	4.79	9.94	2.81	17.46	530.6
18	18	12	59.45	54.40	45.15	42.46	4.90	9.44	2.65	16.95	510.9
18	18	24	59.30	54.45	44.81	42.13	4.86	9.94	2.65	17.28	529.6
18	18	36	60.11	54.67	46.04	41.40	4.63	9.09	4.58	18.05	535.7
18	24	12	59.15	54.62	44.76	42.11	4.69	9.94	2.83	17.29	513.5
18	24	24	58.81	54.50	44.81	41.94	4.80	9.85	2.86	17.47	525.2
18	24	36	59.77	54.48	44.81	41.93	4.82	10.03	2.85	17.48	531.8
Control			59.04	54.52	45.19	42.61	4.77	9.53	2.54	16.77	501.0

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Adjusted for set weight (59.25 g).

Table 4-4. Probability values of orthogonal contrasts for characteristics of broiler breeder eggs exposed to cold stress during incubation versus a group incubated under normal conditions, Experiment 1

Orthogonal contrasts[2]	Transfer weight	Chick weight		Weight loss[1]				Hatch time
		Hatch	Process	1	2	3	Total	
Control v all others	.642	.161	.127	.642	.152	.285	.132	.000[3]
Age linear	.041	.762	.154	.041	.865	.096	184	.0003
Age quadratic	.832	.052	.582	.832	.087	.113	.582	.065
Temperature 18 v 24	.682	.012	.665	.682	.065	.352	.762	.001
Hour linear	.027	.725	.001	.027	.001	.001	.001	.000
Hour quadratic	.471	.072	.472	.471	.192	.662	.562	.000

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] P < .001.

Table 4-5. Probability values from least squares analysis of variance for characteristics of White Leghorn eggs exposed to cold stress during incubation, Experiment 2

Effect[2]	d.f.	Transfer weight	Chick weight		Weight loss[1]				Hatch time
			Hatch	Process	1	2	3	Total	
Age	2	.562	.007	.511	.562	.005	.092	.511	.277
Hour of exposure (HS)	1	.072	.400	.003	.072	.010	.225	.003	.000[4]
Age x HS	2	.873	.594	.443	.873	.939	.681	.443	.003
Location (LOC)[3]	2	.031	.071	.407	.031	.541	.520	.407	.323
Age x LOC	4	.723	.138	.149	.723	.182	.394	.149	.629
HS x LOC	2	.402	.469	.220	.402	.454	.452	.220	.886
Age x HS x LOC	4	.261	.136	.509	.261	.270	.453	.509	.384
Set weight	1	.000	.000	.000	.000	.000	.000	.000	.010
Error term	292								

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] Location in the incubator from top to bottom.

[4] $P < .001$.

Table 4-6. Least squares means for characteristics of White Leghorn eggs exposed to cold stress during incubation versus a group incubated under normal conditions, Experiment 2

	Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				Hatch time (hour)
Effect[2]	Set	Transfer	Hatch	Process	1	2	3	Total	
Age x Hour									
12 24	60.05	54.94	44.34	39.29	5.80	10.05	5.04	20.95	522.3
12 48	61.12	55.08	44.00	38.90	5.66	10.83	5.09	21.35	552.1
14 24	61.23	54.86	44.04	39.41	5.88	10.35	4.62	20.84	526.1
14 48	60.14	55.05	43.74	38.45	5.69	10.94	5.29	21.80	547.3
16 24	61.26	55.02	43.63	39.29	5.72	10.94	4.34	20.96	529.7
16 48	61.00	55.11	42.75	38.17	5.63	11.80	4.57	22.08	548.6
Control	60.34	54.47	44.31	41.84	6.27	9.66	2.46	18.40	502.2
Location[3]									
1	60.85	55.01	43.68	39.08	5.73	10.80	4.59	21.16	530.6
2	61.13	55.02	44.27	39.66	5.72	10.45	4.61	20.59	534.2
3	60.23	54.78	43.53	39.27	5.96	10.71	4.26	20.98	533.0

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Adjusted for set weight (60.75 g).

[3] Order in the incubator from top to bottom.

Table 4-7. Probability values of orthogonal contrasts for characteristics of White Leghorn eggs exposed to cold stress during incubation versus a group incubated under normal conditions, Experiment 2

Orthogonal contrasts[2]	Transfer weight	Chick weight		Weight loss[1]				Hatch time
		Hatch	Process	1	2	3	Total	
Control v all others	.000[3]	.030	.000	.000	.000	.000	.000	.000
Age linear	.592	.008	.248	.592	.001	.051	.248	.210
Age quadratic	.370	.552	.681	.370	.430	.582	.681	.417
Hour 24 v 48	.085	.092	.062	.085	.008	.285	.062	.062

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] $P < .001$.

Table 4-8. Probability values from least squares analysis of variance for characteristics of broiler breeder eggs exposed to cold stress during incubation, Experiment 3

			Chick weight		Weight loss[1]				
Effect[2]	d.f.	Transfer weight	Hatch	Process	1	2	3	Total	Hatch time
Age	2	.375	.863	.089	.375	.440	.019	.089	.004
Hour (HS)	1	.335	.146	.831	.335	.694	.103	.831	.000[3]
Age x HS	2	.016	.428	.003	.016	.727	.026	.003	.027
Incubator (INC)	2	.000	.140	.000	.000	.036	.003	.000	.000
Age x INC	4	.003	.302	.617	.003	.187	.320	.617	.288
HS x INC	2	.005	.182	.026	.005	.146	.223	.026	.788
Age x HS x INC	4	.145	.627	.706	.145	.184	.080	.706	.749
Set weight	1	.000	.000	.000	.000	.000	.097	.000	.010
Error term	781								

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] $P < .001$

Table 4-9. Least squares means for characteristics of broiler breeder eggs exposed to cold stress during incubation versus a group incubated under normal conditions, Experiment 3

	Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				
Effect[2]	Set	Transfer	Hatch	Process	1	2	3	Total	Hatch time (hour)
Age x Hour									
8 24	70.70	64.83	53.24	50.50	6.24	11.72	2.85	20.64	517.6
8 36	71.29	64.97	53.23	51.08	6.10	11.89	2.14	20.06	534.3
12 24	70.93	64.81	53.40	50.37	6.26	11.59	3.03	20.78	516.7
12 36	71.47	64.87	53.26	50.49	6.21	11.63	2.89	20.65	530.1
16 24	70.81	65.24	53.51	50.76	5.84	11.73	2.74	20.38	517.6
16 36	71.27	64.76	53.08	50.14	6.31	11.66	2.94	21.00	530.2
Control	71.29	64.70	53.50	50.98	6.37	11.29	2.51	20.16	496.1
Incubator									
1	71.29	65.06	53.46	50.80	6.01	11.63	2.66	20.34	521.1
2	70.65	65.01	53.27	50.86	6.07	11.86	2.50	20.28	521.7
3	71.29	64.58	53.22	50.19	6.49	11.45	3.03	20.95	518.4

[1] Weight loss 1 = set - transfer, loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Adjusted for set weight (71.08 g).

Table 4-10. Probability values of orthogonal contrasts for characteristics of broiler breeder eggs exposed to cold stress during incubation versus a group incubated under normal conditions, Experiment 3

Orthogonal contrasts[2]	Transfer weight	Chick weight		Weight loss[1]				Hatch time
		Hatch	Process	1	2	3	Total	
Control v all others	.090	.194	.020	.090	.005	.065	.020	.000[3]
Age linear	.502	.578	.022	.502	.180	.004	.022	.015
Age quadratic	.268	.651	.210	.268	.257	.037	.210	.061
Hour 24 v 36	.331	.331	.331	.331	.685	.102	.331	.097
Incubator 1 v 3	.046	.562	.008	.046	.004	.007	.008	.017
Incubator 2 v 1, 3	.001	.098	.002	.001	.168	.015	.002	.001

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] P < .001.

Table 4-11. Probability values from least squares analysis of variance and orthogonal contrasts for characteristics of broiler breeder eggs exposed to cold stress during incubation v a group incubated under normal conditions, Experiment 4

Effect[2]	d.f.	Transfer weight	Chick weight		Weight loss[1]				Hatch time
			Hatch	Process	1	2	3	Total	
Treatment[3]	2	.530	.173	.000[4]	.535	.001	.000	.009	.000
Location (LOC)[5]	4	.930	.254	.149	.930	.094	.208	.149	.073
Treatment x LOC	8	.365	.565	.255	.365	.178	.254	.255	.037
Set weight	1	.000	.000	.000	.000	.000	.000	.000	.058
Error term	165								
Orthogonal contrasts									
Cold v control	1	.159	.084	.015	.159	.000	.190	.015	.000
Cold 6 v 12 h	1	.792	.445	.003	.792	.245	.000	.003	.000
LOC 3 v 1,2,4,5	1	.623	.562	.219	.623	.157	.264	.219	.455
LOC 1,2 v 4,5	1	.788	.082	.022	.788	.130	.285	.022	.447
LOC 1 v 2	1	.964	.145	.795	.964	.057	.069	.795	.141
LOC 4 v 5	1	.908	.968	.814	.908	.585	.746	.814	.022

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] Room temperature (24 C). Total exposure = 48 hours.

[4] $P < .001$.

[5] Order in the incubator from top to bottom.

Table 4-12. Least squares means for characteristics of broiler breeder eggs exposed to cold stress during incubation versus a group incubated under normal conditions, Experiment 4

	Egg weight (g)		Chick weight (g)		Weight loss (g)[1]				
Effect[2]	Set	Transfer	Hatch	Process	1	2	3	Total	Hatch time (hour)
Treatment[3]									
Control	70.74	62.18	51.71	50.13	8.52	10.52	1.58	20.69	497.6
6 h every 48 h[4]	70.34	62.45	51.20	49.08	8.25	11.40	2.11	21.74	534.1
12 h every 96 h	71.33	62.52	51.41	49.95	8.18	11.13	1.45	20.87	539.6
Location[5]									
Location 1	71.19	62.37	51.43	50.05	8.33	11.09	1.38	20.77	525.6
Location 2	69.91	62.38	51.94	51.14	8.31	10.54	1.79	20.47	523.3
Location 3	70.91	62.28	51.30	49.40	8.42	11.32	1.89	21.17	523.1
Location 4	71.18	62.46	51.27	49.55	8.24	11.20	1.72	21.27	521.9
Location 5	70.83	62.42	51.26	49.46	8.28	11.04	1.79	21.36	525.4

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Adjusted for set weight (70.70 g).

[3] Room temperature (24 C). Total exposure= 48 hours.

[4] Hours.

[5] Order in the incubator from top to bottom.

Table 4-13. Probability values from least squares analysis of variance for characteristics of broiler breeder eggs exposed to cold stress during incubation, Experiment 5

			Chick weight		Weight loss[1]				
Effect[2,3]	d.f.	Transfer weight	Hatch	Process	1	2	3	Total	Hatch time
Cross	9	.009	.390	.097	.009	.012	.014	.097	.028
Age	1	.719	.948	.159	.719	.876	.169	.159	.188
Hour (HS)	1	.039	.001	.000[4]	.039	.000	.000	.000	.000
Cross x HS	9	.989	.120	.393	.989	.250	.684	.393	.020
Cross x Age x HS	9	.364	.243	.020	.364	.719	.039	.020	.000
Incubator (INC)	1	.000	.000	.000	.000	.000	.000	.000	.000
Cross x INC	9	.199	.392	.036	.199	.040	.618	.036	.095
Age x INC	1	.809	.024	.036	.809	.155	.448	.036	.157
Cross x Age x INC	9	.911	.008	.056	.911	.033	.206	.056	.001
HS x INC	1	.336	.355	.174	.336	.212	.912	.174	.017
Cross x HS x INC	9	.370	.033	.205	.370	.020	.163	.205	.000
Cross x Age x HS x INC	9	.849	.098	.442	.849	.443	.126	.442	.000
Set weight	1	.000	.000	.000	.000	.000	.000	.000	.042
Error term	872								

[2] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] Interactions that are not significant are not shown (Cross x Age, Age x HS, Age x HS x INC)

[4] P < .001.

Table 4-14. Probability values of orthogonal contrasts for characteristics of broiler breeder eggs exposed to cold stress during incubation, Experiment 5

Orthogonal contrasts[2]	Effect	Transfer weight	Chick weight		Weight loss[1]				Hatch time
			Hatch	Process	1	2	3	Total	
Male A v all others	Cross	.678	.973	.139	.678	.916	.180	.139	.226
Female 3 v 1,2,4,5	Cross	.748	.195	.568	.748	.416	.030	.568	.041
Female 1,2 v 4,5	Cross	.032	.413	.391	.032	.765	.287	.391	.005
Female 1 v 2	Cross	.001	.202	.067	.001	.001	.449	.067	.982
Female 4 v 5	Cross	.593	.397	.018	.593	.936	.021	.018	.715
Age 12 v 16	Age	.719	.984	.159	.719	.876	.169	.159	.188

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set- process.

[2] Set weight used as covariable for other responses.

Table 4-14--continued

Orthogonal Contrasts[2]	Effect	Transfer weight	Chick weight		Weight loss[1]				Hatch time
			Hatch	Process	1	2	3	Total	
Male A v all others x Age	Cross x Age	.890	.287	.324	.890	.320	.124	.324	.212
Female 3 v 1,2,4,5 x Age	Cross x Age	.302	.126	.367	.302	.172	.903	.367	.523
Female 1,2 v 4,5 x Age	Cross x Age	.140	.639	.078	.140	.315	.100	.078	.010
Female 1 v 2 x Age	Cross x Age	.671	.058	.152	.671	.006	.838	.152	.220
Female 4 v 5 x Age	Cross x Age	.091	.786	.320	.091	.146	.120	.320	.026
Hour 24 v 48	Hour	.039	.001	.000[3]	.039	.000	.000	.000	.000

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] $P < .001$.

Table 4-14--continued

Orthogonal Contrasts[2]	Effect	Transfer weight	Chick weight		Weight loss[1]				Hatch time
			Hatch	Process	1	2	3	Total	
Male A v all others x Hour	Cross x Hour	.467	.563	.434	.467	.386	.284	.434	.261
Female 3 v 1,2,4,5 x Hour	Cross x Hour	.973	.053	.418	.973	.191	.425	.418	.666
Female 1,2 v 4,5 x Hour	Cross x Hour	.488	.316	.400	.488	.424	.304	.400	.877
Female 1 v 2 x Hour	Cross x Hour	.467	.106	.034	.467	.045	.631	.034	.021
Female 4 v 5 x Hour	Cross x Hour	.507	.077	.846	.507	.248	.065	.846	.390
Age x Hour	Age x Hour	.595	.612	.522	.595	.342	.335	.522	.579
Incubator 1 v 2	INC	.000[3]	.000	.000	.000	.000	.000	.000	.000

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.
[2] Set weight used as covariable for other responses.
[3] $P < .001$.

Table 4-14--continued

Orthogonal Contrasts[2]	Effect	Transfer weight	Chick weight		Weight loss[1]				Hatch time
			Hatch	Process	1	2	3	Total	
Male A v all others x INC[3]	Cross x INC	.351	.401	.053	.351	.898	.581	.053	.289
Female 3 v 1,2,4,5 x INC	Cross x INC	.971	.710	.115	.971	.975	.250	.115	.918
Female 1,2 v 4,5 x INC	Cross x INC	.032	.977	.024	.032	.670	.128	.024	.014
Female 1 v 2 x INC	Cross x INC	.073	.196	.349	.073	.026	.720	.349	.903
Female 4 v 5 x INC	Cross x INC	.759	.391	.459	.759	.125	.929	.459	.514
Age x INC	Age x INC	.809	.024	.036	.809	.153	.448	.036	.157
Hour x INC	Hour x INC	.336	.355	.174	.336	.212	.912	.174	.017

[1] Weight loss 1 = set - transfer, weight loss 2 = transfer - hatch, weight loss 3 = hatch - process, weight loss total = set - process.

[2] Set weight used as covariable for other responses.

[3] Incubator.

the embryos were cooled was significant in experiments 1 and 3 (Tables 4-1 and 4-13). The third order interaction was highly significant.

Embryonic Mortality

Data concerning embryonic mortality are shown in tables 4-15 through 4-24. Total embryonic mortality for control group was higher in experiments 1, 3, and 4 than in experiment 2 (Tables 4-17, 4-19, 4-21, and 4-22). This could be a consequence of different pre-incubation holding conditions, genetic strain or egg source (Wilson, 1991b). A significant curvilinear effect was found in experiments 2 and 3 (Table 4-18 and 4-20) due to age of the embryo, but not in experiment 5 (Table 4-23). The linear effect in experiment 2 was not significant (P = .071) but it was highly significant in experiment 3. Besides differences in the genotype of the animals, these results suggested a higher susceptibility of younger embryos to longer periods of cooling. Hours of exposure were significant when the period was 48 hours. Eggs cooled for 72 hours (experiment 2) had a 100% embryonic mortality.

Cooling eggs during incubation has been suggested as a tool to manipulate hatch time in order to meet commercial hatcheries conveniences and to improve hatchability by reduction of heat stress on the embryo (Sarpong and Reinhart, 1985; Lancaster and Jones, 1988; Tullet, 1990). Lancaster and Jones (1988) suggested a cooling period no longer than 24 hours at 21 C or higher temperatures between 14 and 17 days of incubation for broiler eggs. The embryonic mortality found in the present study for continuous cooling was higher than for the control groups, but it was lower than the control when the eggs where cooled on day 16 at 24 C for 24 hours (Table 4-17) or when the eggs were cooled for 6 hours every other day as in experiment 4 (Table 4-22). A strong interaction of age at which the embryo is cooled with hours of exposure and temperature on embryonic mortality was established by Webb (1987). This is consistent with results obtained in experiments 1 and 2 (Tables 4-15 and 4-18). In experiment 3 no interaction effects were found and the younger the embryo was, the higher the mortality due to cold stress exposure. This disagreed with some results found in the literature (Wilson, 1991b). Moreng and Bryant (1954) indicated that older embryos were more susceptible to cold exposure. Martin and Insko (1935) found early and late critical periods for turkey embryos. Romanoff and Romanoff (1972) noted that older embryos were more susceptible to high temperatures; however younger embryos were more affected by low temperatures. When the cold stress was intermittent (experiment 4), results were consistent with results suggested by Sarpong and Reinhart (1985) for the group cooled every 48 hours, but not for the group cooled every 96 hours, which showed a higher embryonic mortality. These researchers indicated a significantly lower embryonic mortality of eggs cooled for 6 or 12 hours at 16 days of incubation.

Cold stress during incubation delayed hatch time approximately the same as the period of cooling. When the exposure to cold stress was continuous, the cooled chicks had lower body weight as a consequence of a greater weight loss from transfer to process. Embryonic mortality was greater for all the groups cooled compared to the control group, except for eggs cooled at day 16 at 24 C for 24 hours and eggs cooled every 48 hours (every other day in experiment 4). When exposure time was 48 hours unhealed navels and abnormal feathers down were noted (visual observation). A similar situation was reported by Lancaster and Jones (1988), who indicated that the upper limit of cooling time was about 30 hours with no detrimental effects on chick quality. When the eggs were cooled intermittently, the delay in hatch time was 6 hours greater for the group cooled every 96 hours compared to the group cooled every 48 hours. Embryonic mortality was less for eggs cooled every 48 hours.

Results of the current study indicate that an inverse correlation exists between hatch time and chick weight when variability due to egg weight is removed. Chick weight at hatch is lower in chicks from cooled eggs than in chicks from eggs incubated under normal conditions of temperature. Chicks from cooled eggs are more susceptible to dehydration during holding in the hatcher. Hatch time is delayed by cold stress during embryonic development with detrimental effects on embryo viability and chick weight, but these effects are modified by interactions among the factors involved. Future research should be focused on physiological changes that may occur in chicks hatched from eggs cooled during incubation.

Table 4-15. Categorical analysis and orthogonal contrasts of factors involved in total embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 1

Source	d.f.	Chi-Square[1]	Probability
Intercept	1	3977.66	.000[2]
Age	2	20.17	.000
Temperature (TEM)	1	44.47	.000
Hours of exposure (HS)	2	38.23	.000
Age x TEM	2	3.62	.163
Age x HS	4	23.42	.000
TEM x HS	2	24.43	.000
Age x TEM x HS	4	6.27	.180
Orthogonal contrasts[3]			
Age linear	1	11.19	.000
Age quadratic	1	2.08	.149
TEM 18 v 24	1	44.47	.000
HS linear	1	30.33	.000
HS quadratic	1	11.29	.000
Age linear x HS linear	1	9.25	.002
TEM x HS linear	1	23.35	.000
TEM x HS quadratic	1	13.42	.000

[1] CATMOD procedure.

[2] $P < .001$.

[3] Interactions with $P > .20$ not shown (Age x TEM, Age linear x HS quadratic, Age quadratic x HS)

Table 4-16. Embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 1

			Mortality (%)					
Effect	Total eggs	Total hatched	Before exposure		After exposure		Total	
Embryo age (days)			%	n	%	n	%	n
14	410	314	5.85	24	17.56	72	23.41	96
16	409	323	6.11	25	14.91	61	21.03	86
18	408	273	6.57	27	26.28	108	33.09	135
Temperature (C)								
18	613	407	6.33	39	27.11	167	33.61	206
24	614	503	6.03	37	12.05	74	18.08	111
Hours of exposure								
12	417	332	7.43	31	12.95	54	20.38	85
24	407	322	5.37	22	15.37	63	20.88	85
36	403	256	5.71	23	30.77	124	36.48	147

Table 4-17. Embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 1

Effect			Total eggs	Total hatched	Mortality (%)					
					Before exposure		After exposure		Total	
Age x Temperature x Hour					%	n	%	n	%	n
14	18	12	70	56	4.29	3	15.71	11	20.00	14
14	18	24	67	49	5.97	4	20.90	14	26.87	18
14	18	36	67	38	7.46	5	35.82	24	43.28	29
14	24	12	70	59	7.14	5	8.57	6	15.71	11
14	24	24	69	56	4.35	3	14.49	10	18.84	13
14	24	36	67	56	5.97	4	10.45	7	16.42	11
16	18	12	67	50	8.96	6	16.42	11	25.37	17
16	18	24	68	54	7.35	5	13.24	9	20.59	14
16	18	36	69	45	4.35	3	30.43	21	34.78	24
16	24	12	72	58	6.94	5	12.50	9	19.44	14
16	24	24	66	59	4.55	3	6.06	4	10.61	7
16	24	36	67	57	4.48	3	10.45	7	14.93	10

Table 4-17--continued

Effect			Total eggs	Total hatched	Mortality (%)					
					Before exposure		After exposure		Total	
Age x Temperature x Hour					%	n	%	n	%	n
18	18	12	70	55	8.57	6	12.86	9	21.43	15
18	18	24	68	46	4.23	3	26.76	19	32.35	22
18	18	36	67	14	5.97	4	73.13	49	79.10	53
18	24	12	68	54	8.82	6	11.76	8	20.59	14
18	24	24	69	58	5.80	4	10.14	7	15.94	11
18	24	36	66	46	6.06	4	24.24	16	30.30	20
Control			69	56	-	-	-	-	13.04	9

Table 4-18. Categorical analysis and orthogonal contrasts of factors involved in total embryonic mortality of White Leghorn eggs exposed to cold stress during incubation, Experiment 2

Source	d.f.	Chi-Square[1]	Probability
Intercept	1	869.68	.000[2]
Age	2	45.89	.000
Hours of exposure (HS)	1	116.08	.000
Age x HS	2	48.46	.000
Orthogonal contrasts			
Age linear	1	3.25	.071
Age quadratic	1	21.66	.000
HS 24 v 48	1	116.08	.000
Age linear x HS	1	5.48	.019
Age quadratic x HS	1	26.43	.000

[1] CATMOD procedure.

[2] $P < .0001$.

Table 4-19. Embryonic mortality of White Leghorn eggs exposed to cold stress during incubation, Experiment 2

Effect		Total eggs	Total hatched	Total not hatched	Total Mortality (%)
Embryo age (Age)					
12		94	55	39	41.49
14		93	46	47	50.54
16		89	75	14	17.53
Hours of exposure (HS)					
24		138	121	17	12.32
48		138	55	83	60.14
Age x HS					
12	24	47	42	5	10.64
12	48	47	13	34	72.34
14	24	46	40	6	13.04
14	48	47	6	41	87.23
16	24	45	39	6	13.33
16	48	44	36	8	18.18
Control		46	44	2	4.34

Table 4-20. Categorical analysis and orthogonal contrasts of factors involved in total embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 3

Source	d.f.	Chi-Square[1]	Probability
Intercept	1	3170.52	.000[2]
Age	2	27.07	.000
Hours of exposure (HS)	1	1.24	.265
Age x HS	2	3.31	.191
Orthogonal contrasts			
Age linear	1	15.61	.000
Age quadratic	1	3.63	.056
HS 24 v 36	1	1.24	.265
Age linear x HS	1	1.30	.253
Age quadratic x HS	1	.12	.730

[1] CATMOD procedure.

[2] P < .0001.

Table 4-21. Embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 3.

			Mortality (%)					
Effect	Total eggs	Total hatched	Before exposure		After exposure		Total	
Embryo age (days)			%	n	%	n	%	n
8	220	155	1.82	4	22.73	61	24.55	65
12	220	197	.87	2	13.54	31	14.41	33
16	229	196	1.36	3	9.09	20	10.45	23
Hours of exposure (HS)								
24	333	278	1.50	5	15.02	50	16.52	55
36	336	270	1.19	4	18.45	62	19.64	66
Age x HS								
8 - 24	111	81	1.80	2	25.26	28	27.03	30
8 - 36	109	74	1.83	2	30.28	33	32.11	35
12 - 24	114	102	.81	1	9.65	11	10.53	12
12 - 36	115	94	.87	1	17.39	20	18.26	21
16 - 24	108	95	1.85	2	10.19	11	12.04	13
16 - 36	112	102	.89	1	8.04	9	8.93	10
Control	112	103					8.93	8

Table 4-22. Embryonic mortality and orthogonal contrasts of broiler breeder eggs exposed to cold stress during incubation, Experiment 4

Treatment	Total eggs	Total hatched	Total not hatched	Total Mortality (%)
Control	61	56	5	8.19
Exposure every 48 h	61	57	4	6.55
Exposure every 96 h	67	59	8	11.94
Orthogonal contrasts	d.f	Chi-Square[1]	Probability	
Control v 48, 96 h	1	.06	.808	
Hour 48 v 96	1	1.13	.288	

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[1] CATMOD procedure.

Table 4-23. Categorical analysis and orthogonal contrasts of factors involved in total embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 5

Source	d.f.	Chi-Square[1]	Probability
Intercept	1	1751.42	.000[2]
Cross	9	13.46	.142
Age	1	2.04	.153
Cross x Age	9	8.32	.502
Hours of exposure (HS)	1	21.87	.000
Cross x HS	9	4.88	.845
Age x HS	1	6.54	.010
Cross x Age x HS	9	11.46	.245
Orthogonal contrasts			
Male A v all others	1	3.46	.194
Female 3 v 1,2,4,5	1	5.33	.021
Female 1,2 v 4,5	1	2.97	.084
Female 1 v 2	1	2.39	.122
Female 4 v 5	1	2.99	.092
Age x HS	1	6.54	.010

[1] CATMOD procedure.
[2] $P < .001$.

Table 4-24. Embryonic mortality of broiler breeder eggs exposed to cold stress during incubation, Experiment 5

			Mortality (%)					
Effect	Total Eggs	Total Hatched	Before Exposure		After exposure		Total	
Cross (female x male)			%	n	%	n	%	n
1 A	72	57	6.94	5	13.89	10	20.83	15
1 B	74	47	9.46	7	27.03	20	36.49	27
2 A	74	55	6.76	5	18.92	14	25.68	19
2 B	66	47	13.64	9	15.15	10	28.79	19
3 A	79	58	7.59	6	18.99	15	26.58	21
3 C	82	52	13.41	11	23.17	19	36.59	30
4 A	76	46	13.16	10	26.32	20	39.47	30
4 D	66	42	9.09	6	27.27	18	36.36	24
5 A	85	60	5.88	5	23.53	20	29.41	25
5 E	80	51	13.75	11	22.50	18	36.25	29
Age (day)								
12	372	246	7.79	29	25.94	97	33.87	126
16	376	265	11.70	44	17.63	67	28.46	111
Hours of exposure								
24	378	288	11.11	42	12.69	48	23.81	90
48	374	231	7.29	27	31.35	116	38.65	143

CHAPTER 5
LOW INCUBATION TEMPERATURE EFFECTS ON HEMATOLOGY AND GROWTH OF POSTHATCH BROILER CHICKS

Introduction

Thermal stress during the incubation period affects the normal development of the avian embryo altering its metabolism (Tazawa *et al.*, 1988), heart rate (Oppenhein and Levin, 1975; Tazawa and Nakagawa, 1985; Tazawa and Rahn, 1986), hormone production (Decuypere, 1979; Decuypere *et al.*, 1981, 1983; Kuhn *et al.*, 1982) and immune system including the leukocytes profile (Bednarczyk and Coudert, 1984; Allsep *et al.*, 1992).

In adult chickens, the effect of thermal stress on the immunological system is well documented. Much less information is available concerning the effect of cold stress during incubation on the immunological status of the posthatch chicken. A decreased antibody response of animals incubated at low temperature has been indicated (Preda *et al.*, 1972), with important structural and morphological modifications of the lymphoid organs, mainly the bursa of Fabricius and thymus due to modification of their enzymatic activities (Preda *et al.*, 1971).

The effect of cold stress during incubation appears to be linked to the posthatching performance of the chickens. Beneficial effects on growth have been indicated, possibly because of the elimination of the weaker chicks (Buckland, 1969), or because of a compensatory growth effect, which may persist up to the reproduction period (Geers *et al.*, 1983). Carcass characteristics of 7-week-old chicks may not be affected by cold stress during their incubation period (Sarpong and Reinhart, 1985).

Four experiments were conducted to investigate the effect of cooling broiler breeder eggs at 24 C on different days of incubation and for different hours of exposure during the incubation period on 1) differential count of white blood cells of 1-day old chicks, and 2) their growth performance up to 2 weeks of age.

Materials and Methods

Experiment 1

Eggs obtained from a broiler breeder flock (Arbor Acres)[4] were randomly distributed in groups of 15 eggs and assigned to each of 7 treatments. Three replicates per treatment (15 eggs per replicate) were randomly distributed in each of three adjacent incubators and incubated. During the incubation period, the eggs were removed from the incubator and placed in a chamber at a constant temperature of 24 C. They were cooled at three different ages (8, 12, or 16 days) for 24 or 36 hours. A group was incubated under normal incubator condition of temperature (37.5 C) throughout the incubation period in each incubator. After the hatching process was completed, the chicks were removed from the incubators and placed in baskets identified only by treatment. Ten chicks selected at random from each treatment without regard to sex were bled. Blood was drawn from the wing vein, blood smears were prepared and sent the next day to a laboratory[5] to determine number of white blood cells. It was not possible to do a complete differential blood cells analysis because of problems with blood collection.

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[4] Arbor Acres Farm, Inc., Glastonbury, CT 06033.

Experiment 2

Eggs were obtained from the same broiler breeder company as in experiment 1 and randomly distributed in groups of 15 eggs. Each group of eggs was assigned to one of four treatments and placed at random in each of two incubators. Another group was incubated under normal incubation conditions of temperature throughout the incubation period. As in the first experiment, the embryos were cooled at 24 C at two different ages (12 or 16 days of incubation) for 24 or 48 hours in the same chamber used in experiment 1. After the incubation process was completed and the chicks were removed from the incubator, 10 unsexed birds of each treatment were selected at random and bled. The blood was drawn from the wing vein into heparinized syringes and sent to a commercial laboratory[6] for a complete differential blood cell analysis.

Experiment 3

Chicks obtained from experiment 1 were used for a growth trial. Forty chicks for each treatment were selected at random and distributed in 4 groups

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[5] Veterinary Medical Teaching Hospital
University of Florida Health Science Center
P.O. Box 100103, Gainesville, Fl, 32-610-0103.

[6] Doctors & Physicians Laboratory
P.O. Box 499050, Leesburg, Fl, 34749-9050.

(10 chicks per group). The groups were allocated to each of 4 replicates per treatment, weighed, and placed in battery cages located in a temperature controlled room for 2 weeks.

A starter broiler diet (Table 5-1) and water *ad libitum* were provided to the groups throughout the experimental period. Mean values for initial and final body weights were recorded. The difference between these two values was considered as body weight gain. The amount of feed consumed by each replicate was determined as the difference in weight of feed allocated per replicate during the 2 weeks period and the weight of the feed remaining in the feeder the last day. Feed conversion was calculated as the ratio of the feed consumed divided by body weight gain. The variable responses considered were initial body weight (IBW), final body weight (FBG), body weight gain (BWG), feed consumption (FC), feed conversion (FCR), and embryonic mortality.

Experiment 4

Eggs from 10 different genetic crosses (five females lines crossed with 5 different males lines and identified as 1A, 1B, 2A, 2B, 3A, 3C, 4A, 4D, 5A, and 5E, were obtained from the same broiler breeder company as in experiment 1 and 2. The eggs from each cross were divided into groups of 15 eggs each, assigned to each of 5 treatments (2 ages, 2 temperatures plus a control), and incubated under the same conditions as experiment 2. The chicks obtained after incubation were weighed by treatment group and used in a growth trial for a week under the same conditions and management as experiment 3 and considering the same variables.

Statistical Analysis

The data were analyzed using the SAS General Linear Model (GLM) procedure (SAS Institute, 1989). All the experiments were arranged as factorials plus a control group.

Main effects for the factorial structure in experiments 1 and 2 were day of incubation (Age) and hours of exposure (HS). Data of white blood cells, when expressed as percentages, were transformed to square root after .5 had been added to each value (Steel and Torrie, 1980) prior to the analysis. This transformation did not alter interpretation of results of any analysis; so analyses of untransformed data are reported. Because of empty cells in the factorial structure, some of the least squares means could not be estimated; therefore the data were analyzed as a factorial excluding the control group. Least squares means were obtained for each effect and mean comparisons were done by orthogonal contrasts (Steel and Torrie, 1980). To compare the treatments of the factorial versus the control group, an analysis by treatment was performed (Littell *et al.*, 1991). Least squares mean comparisons of treatments including the control group were done by orthogonal contrasts.

Main effects in experiments 3 and 4 were age and HS. In experiment 4, crosses were considered as main effects and the third factor interaction was used as error term. The effects of age and the interaction age x HS were not significant (experiments 3 and 4), nor were crosses and their interactions with the other factors (experiment 4). Thus, a reduced model considering only HS as source of variation with 3 levels of exposure (0, 24 and 36 or 48 hours) was used for statistical analysis. The reduced model made it possible to perform mean comparisons of the cooled groups versus the control by orthogonal contrasts.

Results and Discussion

White Blood Cells

The values of white blood cells (WBC) obtained in this study for the group incubated under normal conditions agreed with values indicated by Leméz (1979) for 1-day old chicks, except for monocytes, which were higher than the .7% previously reported, and eosinophils, which were lower.

Results indicated an effect of cooling the embryo during the incubation period on WBC. In experiment 1 (Table 5-3), the percentage of heterophil cells appeared to be decreased and lymphocytes increased in the cooled chicks compared with the control. However, these differences were not significant (Table 5-2). Monocytes were significantly affected by the linear effect of age. Embryos cooled at 8 days of incubation had the lowest percentage. Monocytes analysis indicated no significant differences between treatments and the control group (Table 5-2). Eosinophils were significantly affected by all the factors involved. There was a highly significant linear effect of age, HS and the interaction age linear x HS (Table 5-2). Thirty six hours of exposure significantly increased the percentage of eosinophils, but when the embryos were cooled at 16 day of incubation, they showed the lowest percentage. Basophil cells were less affected by cold stress.

Results of experiment 2 indicated no significant differences in percentages of red cells. Highly significant difference in total WBC due to HS and the interaction age x HS (Table 5-3) was observed. When the components of WBC were analyzed as percentages of the total WBC, there were no significant differences due to any of the factors involved or their interactions. (Table 5-3). However, the group cooled for 24 hours had a higher percentage than the control group in all the components of WBC, except for heterophil and basophil cells (Table 5-4). When the analysis was done considering the total of the component of WBC, there were significant effects of HS and the interaction age x HS on total heterophils and lymphocytes (Tables 5-4 and 5-5). Basophils were significantly affected only by HS. Monocytes and eosinophils were not affected by any factor or interactions. Embryos cooled for 24 hours had higher total WBC than the control (Table 5-5). This increase was reflected in all the components of the WBC, except in basophil cells. When HS were up to 48 hours the opposite was true, except for total eosinophil cells, which were lower in the control group (Table 5-5). A similar situation was found due to interaction effects. Embryos cooled at 16 days of incubation for 24 hours had the highest circulating WBC; the lowest values was found for the embryos cooled at the same age for 48 hours (Table 5-5).

The percentages of the components of WBC in experiment 1 differed from experiment 2. It was not possible to determine if these differences were because of different experimental procedures, cellular damage of the smears prepared in experiment 1, variability among the animals due to different genotypes, different effects of treatments or different laboratories. Some values obtained for the eosinophil cells in experiment 1, even for the control group, were lower than values obtained in experiment 2 and those reported by Leméz

Table 5-1. Probability values from least squares analysis of variance and orthogonal contrasts for body weight and white blood cells of 1-day old broiler chicks exposed to cold stress during incubation, Experiment 1

Effect	d.f.	Body weight	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Age (days)	2	.589	.707	.899	.056	.000[1]	.090
Hours of exposure (HS)	1	.461	.645	.730	.381	.003	.794
Age x HS	2	.092	.834	.945	.292	.077	.419
Error term	54						
Orthogonal contrasts							
Age linear	1	.362	.407	.686	.024	.000	.071
Age quadratic	1	.627	.982	.829	.416	.530	.205
HS 24 v 36	1	.461	.645	.730	.381	.013	.794
Age linear x HS	1	.193	.800	.749	.275	.021	.436
Age quadratic x HS	1	.080	.586	.928	.258	.482	.605
Control v all others	1	.112	.241	.352	.371	.102	.262

[1] $P < .001$

Table 5-2. Least squares means of body weight and percentages of components of the white blood cells of 1-day old broiler chicks exposed to cold stress during incubation, Experiment 1

Effect	Body weight	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Age (days)	(g)			%		
8	48.69	66.15	23.02	2.95	3.30	4.65
12	47.74	67.60	22.65	4.60	1.75	3.45
16	47.71	68.95	23.45	4.45	.02	3.00
Hours of exposure (HS)						
24	47.72	68.03	23.03	4.33	1.00	3.56
36	48.37	67.10	22.06	3.66	2.53	3.83
Age x HS						
8 - 24	48.22	66.50	23.40	2.60	2.80	4.40
8 - 36	49.17	65.80	24.60	3.30	3.80	4.90
12 - 24	46.30	69.10	23.30	4.40	.10	3.20
12 - 36	49.18	66.10	23.00	3.80	3.40	3.70
16 - 24	48.65	68.50	23.40	4.00	.10	3.10
16 - 36	46.77	69.40	23.50	3.90	.40	2.90
Control	46.20	70.40	20.20	2.90	1.72	4.70

Table 5-3. Probability values from least squares analysis of variance for body weight and blood cells of 1-day old broiler chicks exposed to cold stress during incubation, Experiment 2

	Source of Variation				
Variable	Age of the embryo	Hours of exposure	Interaction	Analysis by treatment	Orthogonal contrasts[1]
Body weight (g)	.967	.904	.680	.797	.221
Red cells (%)	.738	.722	.588	.959	.485
White blood cells	.385	.003	.009	.002	.448
Heterophils (%)	.279	.468	.276	.458	...
Lymphocytes (%)	.710	.225	.264	.473	...
Monocytes (%)	.118	.884	.196	.245	...
Eosinophils (%)	.192	.998	.466	.652	...
Basophils (%)	.324	.479	.913	.738	...
Total heterophils	.868	.038	.008	.017	.382
Total lymphocytes	.186	.003	.085	.007	.625
Total monocytes	.254	.232	.631	.501	.435
Total eosinophils	.472	.336	.753	.752	.325
Total basophils	.696	.044	.390	.234	.262

[1] Comparison of the control group v all others.

Table 5-4. Least squares means of percentage of components of white blood cells of 1-day old broiler chicks exposed to cold stress during incubation, Experiment 2

Effect	White Cells[1]	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
				%		
Age (days)						
12	7116.07	64.88	25.98	2.60	3.33	3.70
16	8073.48	64.84	28.20	4.01	2.83	3.06
Hours of exposure (HS)						
24	9310.39	60.89	29.83	3.78	2.90	2.95
48	5879.17	64.83	24.83	2.29	3.79	3.80
Age x HS						
12-24	7357.14	60.42	29.71	2.78	3.00	4.07
12-48	6175.00	66.33	24.25	2.34	3.36	3.48
16-24	11263.64	61.36	29.00	3.27	2.81	3.54
16-48	4883.33	60.33	27.41	4.75	4.91	2.58
Control	7990.91	64.45	26.00	3.18	2.81	3.54

[1] Total counts per mm^3

Table 5-5. Least squares means of total white blood cells and their components of 1-day old broiler chicks, exposed to cold stress during incubation, Experiment 2

Effect	White Cells	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Age (days)	Total counts per mm^3					
12	7116.07	4626.39	1806.89	185.15	237.27	260.36
16	8073.48	4740.73	2534.06	274.05	228.57	231.07
Hours of exposure (HS)						
24	9310.39	5415.71	2992.37	348.40	303.21	250.68
48	5879.17	3951.42	1348.58	134.90	227.62	217.00
Age x HS						
12 - 24	7357.14	4417.78	2158.21	213.14	262.78	305.21
12 - 48	6175.00	4135.00	1455.58	157.17	211.75	215.50
16 - 24	11263.64	6413.64	3826.54	339.18	343.64	340.64
16 - 48	4883.33	3067.83	1241.58	208.92	243.50	121.50
Control	7990.91	5096.36	2119.27	258.09	214.64	302.54

(1979) for 1-day old chicks even for the control group. No explanation is apparent for this response.

Conflicting results of the effects of cold stress during avian embryonic development on later life have been reported. Preda *et al.* (1971) indicated a lower antibody response of 15 weeks old chicks hatched from eggs incubated at 37 C, compared with chicks of the same age from eggs incubated at 38.5 C. Chronic cold exposure to 7.2 and 24 C of chickens from the day of hatch to 4 weeks of age increased the antibody response in a study reported by Subba Rao and Glick (1977). In recent research by Allsep *et al.* (1992), turkey embryos cooled for 1 hour at 18 C at different ages showed a significant decrease of circulating heterophils, lymphocytes and basophils, which was reflected in a significant reduction of total WBC. Results obtained in the present study indicated that WBC response was affected by the length of the cold stress and the age of the embryo at which it was cooled, although in some cases these effects were not significant. Modulation of the immune response during extreme changes in temperature may be a function of hormonal mechanisms. The avian immune system is very sensitive to glucocorticoids (Glick, 1984). In adult chickens, stress by exposure to extreme low temperatures causes a decrease of adrenal cholesterol (Perek and Bedrak, 1962) and adrenal corticosterone (Nir *et al.*, 1975). Adrenal cholesterol has been considered to be a precursor of adrenal corticosteroids (Sayers, 1950), which are known to be released in the presence of ACTH (Resko *et al.*, 1964) and to produce lymphopenia and heterophilia in the chicken (Glick, 1958). Corticosterone, the principal corticosteroid in birds (Holmes and Phillips, 1976), is synthesized from cholesterol via an ascorbic acid sensitive pathway (Harvey *et al.*, 1984). Exogenous ACTH increases the incorporation of endogenous corticosterone into bursal lymphoid cells and causes lymphopenia (Gould and Siegel, 1981). In the avian embryo, lymphoid organs and differentiation and proliferation of certain leukocytes, can be influenced by neuroendocrine functions and environmental modification during embryonic development (Branislav *et al.*, 1978; Glick, 1984). The thymus and bursa of Fabricius (primary lymphoid organs in the young chick) reach their maximum sizes after hatch (Kendell, 1980; Glick, 1977); therefore it is possible that there exists an increased susceptibility of these organs to environmental modifications. In addition, embryos exposed to low temperatures during their development exhibit a compensatory growth response after the stressor is removed, which is characterized by a higher rate of cell hyperplasia (Geers *et al.*, 1983). This might explain the unexpected increase of WBC of chicks from eggs cooled for 24 hours, for which the cold stress was not detrimental to their WBC profile, compared with chicks from eggs cooled for 48 hours and those not cooled. On the other hand, 48 hours of exposure to cold stress might be long enough to produce the strain required to stimulate an immunosuppressive endocrine response, that could be greater in older embryos, regardless of the cell hyperplasia because of the compensatory growth.

Growth Performance

Results for growth performance in experiments 3 and 4 are shown in Tables 5-6 and 5-7. In experiment 3 no significant differences in FBW between chicks from cooled eggs and the control group were found, although the former chicks gained more weight as a consequence of a higher FC, which was higher and significant different in the chicks from eggs cooled for a 36 hour period (compared with chicks from eggs incubated under normal conditions of temperature (Table 5-8). The latter group showed the best FCR. However, it was not significantly different from the other groups. In experiment 4, there was a highly significant curvilinear effect (Table 5-8) on FBW, BWG and FC. The chicks from eggs cooled for 24 hours were heavier than the chicks from eggs cooled for 48 hours and the control, but they consumed more feed. Chicks from eggs cooled for 48 hours were not significantly different from the control in FBW and BWG, but they were highly significantly different in FC and FCR. As in experiment 1, the best FCR was for the chicks from eggs incubated under normal conditions.

Results obtained for FBW in these experiments are in agreement with previous results reported by Buckland (1969) and Geers *et al.* (1982, 1983). The chicks from eggs cooled up to 36 hours during incubation, had a greater body weight at 2 weeks of age compared with chicks from eggs incubated under normal temperature conditions. However, the former needed more feed to achieve a unit of weight, possibly because of a higher requirement for maintenance (Geers *et al.*, 1982). When the cold stress was extended to 48 hours, the chicks did not show compensatory growth and their FCR at 1 week of age was increased. In terms of FCR, cooling eggs during their embryonic development had detrimental effects on the performance of the chicks up to 2 weeks of age.

Table 5-6. Probability values of least squares analysis of variance, orthogonal contrasts, and least squares means for characteristics of broiler chicks from 1-day old to 2 weeks of age exposed to cold stress during incubation, Experiment 3

Source of variation	d.f.	Initial body weight	Final body weight	Body weight gain	Feed consumed	Feed conversion
Hour of exposure	2	.561	.622	.571	.112	.375
Error term	24					
Orthogonal contrasts						
24 h v control, 36 h	1	.205	.487	.531	.734	.621
Control v 36 h	1	.951	.312	.324	.010	.225
Least squares means (g)						
Control		49.62	345.0	295.3	348.02	1.17
24 hour exposure		50.66	361.8	311.1	369.00	1.19
36 hour exposure		49.58	362.0	312.4	383.75	1.23

Table 5-7. Probability values of least squares analysis of variance, orthogonal contrasts, and least squares means for characteristics of broiler chicks from 1-day old to 1 week of age exposed to cold stress during incubation, Experiment 4

Source of variation	d.f.	Initial body weight	Final body weight	Body weight gain	Feed consumed	Feed conversion
Hour of exposure	2	.227	.009	.002	.006	.018
Error term	47					
Orthogonal contrasts						
24 h v control, 48 h	1	.421	.001	.002	.005	.132
Control v 48 h	1	.187	.991	.871	.020	.009
Least squares means (g)						
Control		48.78	143.1	94.3	92.20	1.03
24 hour exposure		48.72	155.5	106.8	124.10	1.16
48 hour exposure		47.73	141.1	94.1	112.15	1.19

Embryonic mortality due to cold stress was higher than the average embryonic mortality during incubation under normal conditions, but mortality in both experiments 3 and 4 was less than 5% (data not reported), which might agree with the suggestion of Buckland (1969) that cold stress eliminates the weaker and potentially slower growing birds.

Results obtained in the current study indicated that 24 hours of exposure to cold stress during the incubation period of broiler breeder eggs, enhanced WBC profile of one-day old chicks with a detrimental effect afterward. It is unknown whether the antibody response of the chicks could be related to their WBC profiles. Feed convection of the up to 2 weeks of age was adversely affected by the exposure to cold stress during their embryonic development, although mortality was lower than mortality reported in the literature for broiler chicks during the first 2 weeks of age.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Genotype and age of the breeder, egg characteristics and incubation temperature are among the factors which affect incubation, specially the length of the incubation period and the characteristics of the chick before, at hatch, and after hatch. How these factors may influence the incubation process and the posthatch performance of the chick is not clear.

Firstly, the effects of genotype and age of the breeders on incubation characteristics and egg components were evaluated in 2 experiments. Broiler breeder eggs from different strains (identified as A, B, C, D and E in experiment 1; 1, 2, 3, 4, 5, and 6 in experiment 2) at three different ages were incubated under normal conditions of temperature (37.5 C). Initial egg weight (set weight), egg weight at 456 hours of incubation (transfer weight), chick weight at hatch (hatch weight), chick weight at 516 hours, when the chick was removed from the incubator (process weight), weight loss (differences between set, transfer, hatch and process weight), hatch time, total hatchability (experiment 1) and mortality (experiment 2), as well as yolk weight, albumen weight, shell weight, and yolk:albumen ratio (Y:A) in experiment 2, were recorded and analyzed as a factorial with strain and age as fixed main effects. Least squares means for the effects were estimated and compared by orthogonal contrasts.

To reduce the effect of set weight on the variables studied, this variable was used as covariable in the least squares analyses. Data of yolk weight, albumen weight, and shell weight were expressed as percentages of initial egg weight.

The analysis detected a highly significant effect of set weight ($P < .001$) on transfer and hatch weights. This effect was reduced in weight losses ($P < .010$) and hatch time ($P = .018$ and .094 in experiments 1 and 2. The same relationship between set weight and the other variables was observed with Pearson correlation coefficients.

The effects of strain and the interaction strain x age were highly significant on all variables of interest in experiment 1. Orthogonal comparisons of main effects indicated that strain A and B did not differ in any of the variables considered. In experiment 2, strain did not affect significantly the response variables. A highly significant linear response due to age was found for transfer, hatch and process weights, while the quadratic response was only significant for hatch weight ($P = .001$) in experiment 1. Hatch time exhibited a highly significant curvilinear effect of age ($P = .001$) in experiment 1. Hatch weight and hatch time were highly significantly affected by the incubators used in experiment 1 ($P = .001$). In experiment 1 probability values for significance of the effects were lower than in experiment 2. This may be because of larger genetic differences among strains studied.

Total hatchability (experiment 1) and embryonic mortality (experiment 2) were significantly affected by strain, age, and the interaction strain x age, although in experiment 2 only strain 2 was significantly different from the others.

Egg component results indicated significant differences due to strain, age and the interaction strain x age. Yolk:albumen ratio increased linearly with age, as a consequence of a linear decrease of albumen weight. No significant effects were found for shell weight.

The effect of continuous (experiments 1, 2, 3, and 5) or intermittent cold stress (experiment 4), and the genotype of the breeder (experiment 5) on incubation process were evaluated. Broiler breeder and White Leghorn breeder eggs were set to be incubated and cooled (18 or 24 C in experiment 1, and 24 C in the others) at different ages of the embryo (8, 12, 14, 16, or 18 days) and different hours of exposure (12, 24, 36, 48, or 72 hours). Set weight was recorded. At the age selected to apply the cold stress, the eggs were removed from the incubator and placed in a chamber at a constant temperature or at hatchery room temperature (23 C). After the hours of exposure (HS) to cold stress selected for each treatment, the eggs were returned to the incubator. A control group was incubated under normal conditions of temperature in each experiment. Response variables considered were the same as in the first 2 experiments. Process weight was delayed from that of control equivalent to the hours the eggs were cooled. Embryonic mortality was classified as before and after treatment of cold stress. The data were analyzed 1) as a factorial without the control group to estimate least squares means for main effects and 2) by treatments, considering as treatment combination of temperature, age, and hours of exposure plus a control. Set weight was considered as a covariable. None of the eggs cooled for 72 hours hatched; thus they were not included in the statistical analyses. When more than 1 incubator was used, incubator was considered as a source of variation.

The effect of cold stress during incubation on embryo development depended upon the age at which the embryo was cooled, hours of exposure (HS), temperature (TEM), genotype of the breeders (crosses), and incubator conditions. At transfer the cooled eggs were heavier than the control as a consequence of a lower weight loss, possibly because of a reduced metabolic rate of the cooled embryo, but these differences were only significant in experiment 2. A significant genotype x environment interaction was evident for this variable (experiment 5). Hatch weight was more sensitive to the effects of the factors involved than transfer weight. It was affected by temperature, age of the embryo and the interaction TEM x age (experiments 1 and 2). Hours of exposure had a detrimental effect on hatch weight when it was up to 48 hours (experiments 2 and 5). The control group was heavier at hatch than the other groups, except for some second and third order interactions where incubator effects were involved (experiments 1 and 5). Process weight was heavier for the control group, although these differences were not significant in most of the cases. Weight loss total was greater for all the cooled chicks. This indicated more susceptibility to dehydration from transfer to process of the cooled chicks compared with the control.

The major effects of cold stress during incubation was on hatch time and embryonic mortality. An inverse relationship was evident between hatch time and chick weight. Hatch time was delayed in all the eggs cooled. The longer the exposure period, the longer the hatch time, but in general the delay was shorter than the respective exposure to cold stress and was significantly affected by all the factors involved, including genotype of the breeders. Mortality of embryos exposed to continuous cooling was higher than the control group, but it was lower for embryos cooled on day 16 at 24 C for 24 hours and for eggs cooled for 6 hours every other day.

Four experiments to investigate the effect of cooling broiler breeder eggs during incubation on differential count of blood cells of 1-day-old chicks and their growth performance up to 2 weeks of age were conducted. The eggs were cooled as described before. They were cooled at different ages and for different hours (8, 12, or 16 days for 24 or 36 hours in experiment 1; 12 or 16 for 24 or 48 hours in experiment 2). A control group was incubated under normal conditions of temperature. After process weight, 10 chicks per treatment were selected at random and bled. Blood samples were collected, blood smears were prepared and sent to a laboratory to determine components of white blood cells (experiment 1) and complete differential blood cells analysis (experiment 2). Chicks obtained from these experiments were used to test their growth performance up to 1 and 2 weeks (experiments 4 and 3). They were allocated to each of four 10-bird replicates per treatment, weighed and placed in battery cages located in a temperature controlled room. Starter broiler diet and water were provided *ad libitum*. The statistical analyses for experiments 1 and 2 were as described before for the experiments testing cold stress during incubation. For growth trials, a reduced model with hours of exposure (HS) as a source of variation was used.

Total white blood cells (WBC) of 1-day-old chicks were altered significantly by HS and the interaction age X HS. This effect was evident in total heterophils and lymphocytes which were increased for 24 HS, but decreased for 48 HS compared with values obtained for the control. Control groups were not statistically different from cooled groups. When blood cells were analyzed as percentages of WBC, percentage of eosinophils was affected by all the factors and their interactions in experiment 1, but not in experiment 2. No explanation was apparent for this response, although differences in experimental procedure, cellular damage, variability due to genotype of the chicks, or treatment effects may have existed.

In the avian embryos lymphoid organs and differentiation and proliferation of certain leukocytes may be influenced by neuroendocrine functions and environmental modifications during embryonic development. In adult chicks the immune response is very sensitive to the action of glucorticoids, which may lead to lymphopenia and heterophilia in extreme low temperature conditions. Furthermore, embryos exposed to low temperatures during their development exhibit compensatory growth response after the stressor is removed, which is characterized by a higher rate of cell hyperplasia. It is possible that a higher rate of cell hyperplasia in chicks cooled up to 36 hours during their incubation occurred due to compensatory growth response, which might explain the increase of total WBC in these chicks. However, when the embryos were cooled for 48 hours, the stress might have been long enough to stimulate an immunosuppressive endocrine response of the animals.

The effect of cold stress during incubation on growth performance of the chicks was similar to the effect in total WBC. Final body weight was greater for chicks cooled up to 36 hours and lower in chicks cooled for 48 hours when compared with the control, but the control group had a better feed conversion. These differences were highly significant between the control and chicks from eggs cooled for 48 hours.

Results obtained in the current study indicate that, regardless of differences in egg weight, some strains perform differently in egg and chick characteristics during the incubation process, and hatch time. This response possibly could be because of differences in egg components. These differences may change with age of the hens and apparently are not related to total hatchability or embryonic mortality.

Effect of cold stress during incubation on chick characteristics depend on whether the stress is continuous or intermittent, age at which the embryo is cooled, length of the exposure, interactions between age and hours of exposure, and genotype of the breeders. Continuous cooling is more deleterious than intermittent. Chick weight at hatch is lower in chicks from egg cooled than in chicks from eggs incubated under normal conditions of temperature. Chicks from cooled eggs are more susceptible to dehydration during holding time, and consequently may have lower weights at process. Hatch time is delayed by cold stress during embryonic development, and is inversely correlated with chick weight at hatch. Embryo viability is detrimentally affected by cold stress during incubation.

Cold stress during incubation alters total white blood cells of 1-day old chicks. Exposure of 24 hours enhances the white blood cells profile, but exposure up to 48 hours is detrimental. Whether or not this response may influence immunological response of the chicks in their later life is unknown.

Growth performance up to 2 weeks of age of chicks from eggs cooled during incubation is not affected. However, they need more feed to gain a unit of weight than chicks from eggs incubated under normal conditions of temperature.

Exposure of continuous cold stress for 48 hours has a significant detrimental effect on feed conversion during the first week of age of chicks from eggs cooled during incubation.

Commercial strains of chicken differ in their performance during the incubation process. This performance is modified by environmental factors such as age of the female and temperature during incubation that indicates significant genotype x environment interactions. This strongly suggests that genotype of the female breeder should be considered when environmental conditions of incubation to approach optimum hatch performance and chick quality are recommended.

Cooling the eggs during incubation should be evaluated in terms of an increased embryonic mortality, reduction of the number of chicks per year due to a longer incubation period, and possibly detrimental effects on white blood cells and feed conversion of posthatch chicks. Also, development of new management strategies, principally the time the chicks will be held in the hatcher should be considered.

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BIOGRAPHICAL SKETCH

María Elena Suárez Oporta was born in Juigalpa, Chontales, Nicaragua on August 13, 1949. She attended elementary, secondary and high schools at Juigalpa, Chontales, Nicaragua, graduating from Liceo Agrícola de Chontales, in 1968. She attended the Facultad de Ciencias Agropecuarias at the Universidad Centroamericana, Managua, Nicaragua, earning a degree of Licenciada en Zootecnia in October 27, 1980. In 1982, she went to Centro de Ganadería, Colegio de Postgraduados at Chapingo, Mexico, to pursue her Maestro en Ciencias degree, which she obtained in December 13, 1984, presenting the thesis "Interaction Genotipo x Ambiente en Líneas Comerciales de Pollos de Engorda." In January, 1985, she began working at Centro de Ganadería, Colegio de Postgraduados at Chapingo, México, mainly working on Poultry Animal Breeding.

She is married to Carlos Miguel García-Bojalil, and they have a daughter, Carla Denisse, born October 14, 1986.

In January, 1990, she began her doctoral program in the Department of Animal Science, under the supervision of Dr. Henry R. Wilson in the area of poultry physiology.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Henry R. Wilson

Henry R. Wilson, Chairman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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